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# PROCESS OF RAISING SQUALENE LEVELS IN PLANTS AND DNA SEQUENCES USED THEREFOR

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**Abstract**

The invention provides DNA that can be introduced into the genomes of plants to produce genetically-modified plants having higher levels of squalene than the natural plants. The DNA corresponds to squalene epoxidase gene of the same or a related plant, and may have the sequence as shown by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 or SEQ ID NO:11; or a part of such a sequence or a sequence having at least 60 % homology with such a sequence. The DNA is introduced into the genome in a way that results in down-regulation of an exogenous plant squalene epoxidase gene to suppress the expression of squalene epoxidase. The invention also relates to a process of producing genetically-modified plants, plasmids and vectors used in the method, genetically-modified plants and seeds thereof, and a method of producing squalene from the modified plants.

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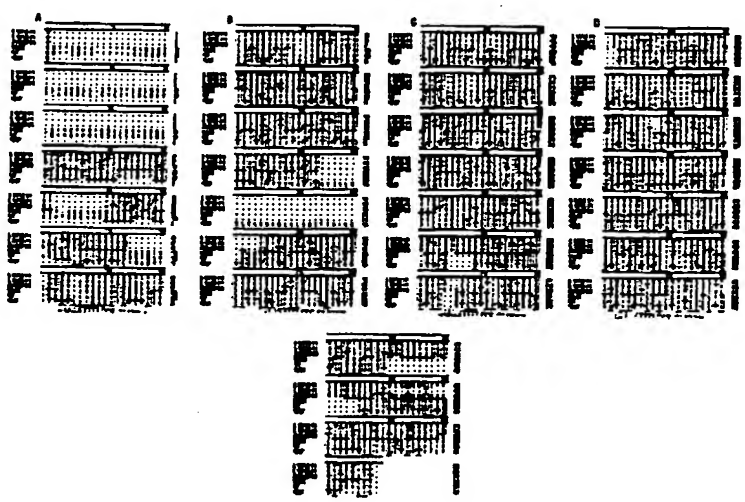

(57) Abstract

The invention provides DNA that can be introduced into the genomes of plants to produce genetically-modified plants having higher levels of squalene than the natural plants. The DNA corresponds to squalene epoxidase gene of the same or a related plant, and may have the sequence as shown by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 or SEQ ID NO:11; or a part of such a sequence or a sequence having at least 60 % homology with such a sequence. The DNA is introduced into the genome in a way that results in down-regulation of an exogenous plant squalene epoxidase gene to suppress the expression of squalene epoxidase. The invention also relates to a process of producing genetically-modified plants, plasmids and vectors used in the method, genetically-modified plants and seeds thereof, and a method of producing squalene from the modified plants.

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TITLE: PROCESS OF RAISING SQUALENE LEVELS IN PLANTS AND DNA SEQUENCES USED THEREFOR

TECHNICAL FIELD

This invention relates to the production of squalene for commercial and industrial uses. More particularly, the invention relates to a process by which natural squalene levels in plants can be increased, and to nucleotide sequences that can be introduced into plants to cause the desired increase, and plasmids, vectors, etc., useful in the process.

BACKGROUND ART

There is a US$ 125 million per annum market for squalene, a colourless oil used in the cosmetics and health industries (Kaiya, 1990). Squalene is currently obtained mainly from shark liver, but it also occurs in small quantities in vegetable oils. Squalene extracted from shark liver is declining in supply (Kaiya 1990) and the harvesting of sharks for this purpose is anyway environmentally unfriendly and is becoming less acceptable as environmental concerns increase in society.

Squalene can be extracted from olive oil, although the amounts are not sufficient to supply even the cosmetics market (Bondioli et al. 1992; Bondioli et al. 1993). Squalene could be extracted from other vegetable oils, but the levels of the hydrocarbon in the oil are too low for this to be economically viable. There are at present no Canadian crops used for squalene production. It has been suggested that, if the levels of squalene occurring in oilseeds could be increased, the traditional source of squalene could be replaced by oilseed crops, to the benefit of both the environment and those countries, such as Canada, that grow crops of this kind in abundance. Many vegetable oils undergo deodorization by vacuum distillation as a routine part of refining. Most of the squalene in the oil can be recovered in the deodorizer distillate which is a by-product of this

process (Bondioli et al., 1993). Typically, squalene is concentrated more than one hundred fold in the deodorizer distillate relative to the levels in unrefined vegetable oils. For commercial viability, vegetable oil deodorizer distillates should contain at least 5% (w/w) squalene. Currently, soybean and canola deodorizer distillates contain squalene in the 0.1-3% range (Ramamurthi, S., 1994). Consequently, an increase of two-fold or more in the squalene content of these oilseeds could result in commercially viable squalene production from vegetable oils.

It has been shown that in plant cell cultures, squalene accumulates in the presence of squalene epoxidase inhibitors, e.g. allylamines such as terbinafine (Yates et al. 1991). Apparently, much of the squalene produced in plants is converted to the epoxide by squalene epoxidase, and ultimately to plant sterols. In fact, all plant and higher life forms contain squalene and squalene epoxidase genes, but little squalene accumulates in the tissues of such life forms because of the effects of the expressed squalene epoxidase. Therefore, inhibition of the epoxidase gives squalene an opportunity to accumulate. However, there are as yet no commercial processes based on this concept.

A main problem addressed by the inventors of the present invention is therefore to create a plant crop, particularly an oilseed crop, which accumulates squalene in harvestable tissues, such as seeds, at sufficient levels for commercially-viable extraction.

## DISCLOSURE OF THE INVENTION

An object of the present invention is to provide new sources of squalene that have the potential to be exploited on a commercial basis to replace conventional commercial sources of squalene.

Another object of the present invention, is to generate squalene-producing plants modified to accumulate

squalene in the plant tissue (e.g. in seeds) in sufficient quantities to make the extraction of squalene commercially attractive.

Another object of the invention is to identify squalene epoxidase genes in plants, and to partially or completely neutralise the expression of such genes.

Another object of the invention is to produce DNA clones, constructs and vectors suitable for modifying the genomes of plants to reduce expression of squalene epoxidase.

Yet another object of the invention is to provide a commercial process for producing squalene from plant tissue, especially seeds.

The inventors of the present invention have discovered the DNA sequences of the genes encoding squalene epoxidase (squalene monooxygenase (2,3-epoxidizing); EC 1.14.99.7) from the plants *Arabidopsis thaliana* (thale cress), and *Brassica napus* (rapeseed, canola), as well as a second gene from *Arabidopsis* and one from *Ricinus communis* (castor), and using this knowledge have developed a process of modifying the genomes of such plants to produce genetically-modified plants which accumulate squalene at higher than natural levels. Moreover, the process may be operated to increase squalene levels in plants using DNA based on squalene epoxidase genes from different but related plants.

According to one aspect of the invention, there is provided an isolated and cloned DNA (polynucleotide) suitable for introduction into a genome of a plant to suppress expression of squalene epoxidase by said plant below natural levels, wherein the DNA has a sequence corresponding at least in part to a squalene epoxidase gene of a plant.

The DNA preferably has a sequence corresponding to all or part of a specific sequence selected from SEQ ID

NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10 (as shown in the following Sequence Listing); or having at least 60% (more preferably at least 70%) homology thereto.

The measure of homology between two DNA (polynucleotide) sequences as used in this specification is the similarity index given by application of the Wilbur-Lipman algorithm of the MEGALIGN® computer program (DNASTAR) in aligning and comparing DNA sequences corresponding to a complete polypeptide coding region using the parameters ktuple=3, gap penalty=3 and window=20.

According to another aspect of the invention, there is provided a process of producing genetically-modified plants having increased levels of squalene in tissues of the plants compared to corresponding wild-type plants, wherein the plant genome is modified to suppress expression of squalene expoxidase by said plant. The genome is modified by introducing at least one exogenous DNA sequence that corresponds, at least in part, to one or more endogenous squalene epoxidase genes of the plant.

The DNA sequence introduced into said plant genome has at least 60%, and more preferably at least 70%, homology to said one or more of the endogenous squalene epoxidase genes, and is preferably all or part of a sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10.

According to yet another aspect of the invention, at least in a preferred form, there is provided a process of producing genetically-modified plants having increased levels of squalene in tissues of the plants compared to corresponding wild-type plants, wherein the plant genome is modified to suppress expression of squalene expoxidase by said plant, raising squalene levels of a plant, by introducing into the genome of the plant a nucleotide sequence that reduces or prevents expression of squalene

epoxidase. The DNA introduced into the genome includes a transcriptional promoter and a sequence that when transcribed from the promoter is complementary or antisense to all or part of at least one squalene epoxidase messenger RNA produced by the plant.

The invention also relates to plasmids and vectors used in the processes indicated above, and as disclosed later.

The invention further relates to a genetically-modified plant capable of accumulating squalene at levels higher than the corresponding wild-type plant, produced by a process as indicated above, or a seed of such a plant.

The invention additionally relates to a process of producing squalene, which involves growing a genetically-modified plant as defined above, harvesting the plant or seeds of the plant, and extracting squalene from the harvested plant or seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the alignment of deduced amino acid sequences of the clones pDR111 (*B. napus* 111) [SEQ ID NO:4], pDR411 (*B. napus* 411) [SEQ ID NO:11] and 129F12T7 (*Arabidopsis*) [SEQ ID NO:2], and of the known squalene epoxidase genes of mouse (DNA Database of Japan D42048) [SEQ ID NO:6], rat (DNA Database of Japan D37920) [SEQ ID NO:7], and baker's yeast (Genbank M64994) [SEQ ID NO:8]; the alignment was done using the MEGALIGN™ program of the LASERGENE™ suite of programs (DNASTAR) using a multiple alignment gap penalty of 20; and

Figures 2, 3 and 4 are plasmid maps of three vectors (pSE111A, pSE411A and pSE129A, respectively) produced according to one embodiment of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

General Discussion

The concept underlying the present invention is to identify squalene epoxidase genes of oilseed plants (or

possibly other plants, since all plants appear to have genes for the production of squalene, and particularly those plants that are capable of accumulating squalene in their harvestable tissue) and then to use that knowledge to create genetically-modified plants in which the expression of squalene expoxidase is decreased partially or fully compared to the natural level of expression, so that squalene naturally produced by the plants can accumulate in the seeds or other tissue to levels that make extraction commercially attractive.

The approach taken by the inventors of the present invention to identify squalene epoxidase genes of plants was initially to use the DNA sequence of a known squalene epoxidase gene from yeast to identify equivalent genes in suitable plant species, e.g. by heterologous hybridization, on the assumption that all squalene epoxidase genes will have a considerable degree of similarity. Once one or several plant squalene epoxidase genes have been identified in this way, those plant genes can then be used to identify additional squalene epoxidase genes from other plants.

Heterologous Hybridization

Nucleic acid hybridization is a technique used to identify specific nucleic acids from a mixture. Southern analysis is a type of nucleic acid hybridization in which DNA is typically digested with restriction enzymes, separated by gel electrophoresis and bound to a nitrocellulose or nylon membrane. A nucleic acid probe, which is typically radio-labeled or otherwise rendered easily detectable, is hybridized to the bound DNA by exposing it to the membrane-bound DNA under specific conditions and washing any unbound or loosely bound probe away. The location of the bound probe is then detected by autoradiography or other detection method. The

location of the bound probe is an indication that DNA sequences that are similar to those in the probe nucleic acid are present. Hybridization may also be done with DNA of clones of a recombinant DNA library, such as a cDNA library, when that DNA has been bound to a membrane after plating the library out (Ausubel et al., 1994). Of course, the method used by the inventors to identify the genes disclosed in the present application may be used to identify equivalent genes from other plants. As noted above, the process originally used by the inventors to identify the *Arabidopsis* gene was based on further analysis of a gene that was tentatively identified from a publicly available database containing partial sequences (Expressed Sequence Tags or EST's) submitted by other workers from randomly chosen (unidentified) gene clones. EST's from other species (such as rice, castor) can also be searched in the same way to find other possible squalene epoxidase genes present in such plants (depending on the more or less accidental sequencing of the desired genes) using the *Arabidopsis* and *B. napus* sequences disclosed herein.

The inventors have, for example, found other EST's from plants that have tentatively been identified as squalene epoxidase genes by comparing them to the *Ababidopsis* and *B. napus* sequences discussed above. Thus, sequences corresponding to Genbank Accession Numbers T15019 (obtainable from Dr. C.R. Somerville, Carnegie Institution, 290 Panama St., Stanford, CA 94305, USA) and W43353 (obtainable from DNA Stock Center, Arabidopsis Biological Resource Center, Ohio State University, 1060 Carmack Road, Columbus, OH 43210-1002, USA) have been predicted to correspond to squalene epoxidases genes from *Ricinus communis* (castor) and *Arabidopsis* (a second *Arabidopsis* gene).

Perhaps more importantly, the process by which the *B. napus* gene was cloned can be used to clone other plant species. The (heterologous hybridization) methods are well known, but the process requires the knowledge and use of the novel plant squalene epoxidase sequences disclosed in this application.

If the hybridization and washing are done under conditions which are considered stringent (e.g., at relatively high temperature and/or low salt and/or high formamide concentration), then the sequences detected generally have a high degree of similarity to the probe nucleic acid. If hybridization and washing are done at lower stringency, then it is possible to detect sequences that are lower in similarity to the probe. Discussions of this detection of similar sequences by hybridization can be found in Beltz et al. (1983) and Yamamoto and Kadowaki (1995). From the point of view of gene cloning, if one obtains a clone for a gene in one organism, one can use low stringency hybridization of the DNA clones corresponding to a related organism to detect the homologous gene sequences of that organism. As mentioned before, the success of this approach depends on the similarity of the sequences of the homologous genes which in turn generally depends on the evolutionary relationship between the organisms.

Once identified, sequenced and cloned, the DNA of suitable plant species may then be modified or manipulated with any technique capable of decreasing the expression of a natural gene based on an isolated DNA clone corresponding, at least in part, to that gene. Suitable methods, at present, include antisense technologies (Bourque, 1995), co-suppression or gene silencing technologies (Meyer, 1995; Stam et al., 1997; Matzke and Matzke, 1995), and ribozyme technologies (Wegener et al. 1994; Barinaga, 1993).

These technologies are discussed in more detail below.

Down-regulation of Gene expression

General

The activity of a particular enzyme, such as squalene epoxidase, is dependent on, among other things (such as the biochemical environment), the amount of enzyme (usually, and for the sake of this argument, a protein) that is present. The amount of enzyme present depends on the expression of the gene or genes encoding the enzyme of interest. Gene expression usually includes (not necessarily in this order) transcription of DNA to generate RNA, processing of the RNA produced from transcription, transport of RNA to the site of translation, translation of mature messenger RNA into polypeptide, proteolytic processing and folding of the nascent polypeptide, transport of the protein product to various cellular compartments, and post-translational modification of the protein (such as phosphorylation or glycosylation). Any effect or difference in any of the processes involved in gene expression can have an effect on the level of expression of an enzyme encoded by a given gene or genes. Gene expression often varies with cell type, tissue type and developmental stage. Likewise, enzyme levels in different cells and tissues and at different developmental stages varies widely. (For plant nuclear genes, this is often the result of differential transcription.)

Gene expression can also be affected by the breakdown of the gene product, the enzyme, or any of the intermediates in gene expression, such as precursor RNA.

From a genetic engineering point of view, in principle, gene expression can be down-regulated by affecting almost any of the processes involved. For

example, although the mechanism is not well established, antisense technology (as discussed below) decreases the amount of translatable messenger RNA (mRNA) in an organism.

A) Antisense technology

An appropriate antisense technology is disclosed, for example, in US patent 5,190,931 issued on March 2, 1993 to Masayori Inouye. The disclosure of this patent is incorporated herein by reference. In short, this technology can be used to regulate or inhibit gene expression in a cell by incorporating into the genetic material of the cell a nucleic acid sequence which is transcribed to produce an mRNA which is complementary to and capable of binding to the mRNA produced by the genetic material of the cell. The introduced nucleic acid sequences include equivalents of the gene to be regulated, or parts thereof, oriented in antisense fashion relative to a transcriptional promoter. Thus, the squalene epoxidase sequence, or part thereof, is introduced into the genetic material of the cell as a construct positioned between a transcriptional promoter segment and a transcriptional termination segment. The mRNA produced when the antisense sequences are transcribed binds or hybridizes to the mRNA from the squalene epoxidase gene of interest and prevents translation to a corresponding protein. Therefore, the protein coded for by the gene is not produced, or is produced in smaller quantities than would otherwise be the case. By introducing a gene that has a sequence that is antisense to the natural squalene epoxidase gene in oilseed plants, the epoxidation of squalene can be inhibited or reduced so that squalene accumulates in the plant tissues, especially the seeds, which can then be harvested in the usual way and the squalene extracted

using conventional techniques.

In terms of the process of antisense down-regulation of squalene epoxidase genes, for any plant species, it is generally necessary to use a gene from a closely related plant such that the genes are more than about 60%, and preferably about 70%, identical at the DNA level (Murphy, 1996). Thus, homologous (equivalent) genes from the same family of plants, would reasonably be expected to give an antisense effect on any member species of that family. For example, *Arabidopsis* genes have been found to have antisense effects in *B. napus* (Murphy, 1996).

The antisense DNA in expressible form may be introduced into plant cells by any suitable transformation technique, e.g. *in planta* transformation (such as wound inoculation or vacuum infiltration). Transformation may also be carried out by co-cultivation of cotyledonary petioles and hypocotyl explants (e.g. of *B. napus* and *B. carinata*) with *A. tumefaciens* bearing suitable constructs (Moloney et al. (1989) and DeBlock et al.(1989)).

It would, of course, be optimal to identify a natural squalene epoxidase gene for each plant species to be modified in order to ensure complete correspondence of the DNA used to modify the natural gene and the DNA of the natural gene itself. If a gene from one plant species has been cloned, there are methods available to clone the same gene from other plants. The reliability of these methods (heterologous hybridization methods) depends on the similarity of the DNA sequence of the genes. If the DNA sequences have at least 60% of their sequence identical, and more preferably at least 70%, then the methods are usually reliable. Sequence similarity depends mostly on evolutionary (ancestral) relationships between plants. Practically, this means that either of the two genes first cloned by the

inventors (the *Arabidopsis* and *B. napus* genes) may be used to clone the same gene in any other dicotyledonous plant (dicot), including, but not limited to soybean, tobacco, amaranth, potato, cotton, flax, bean, and pea. It is also reasonable to assume that the *Arabidopsis* or *B. napus* genes could also be used to clone the same genes from monocotyledonous plants (monocots), such as wheat, corn and barley.

The antisense effect occurs when hybridization can occur between antisense RNA and native RNA under the conditions prevailing in the cell. This may occur when the antisense RNA (and corresponding cDNA) contains as few as 20 nucleotides. More preferably, however, there should be at least 100 nucleotides in the cDNA to guarantee the required effect, and of course any larger portion up to the entire cDNA may be employed. In short, therefore, for effective antisense technology, the DNA sequence introduced into the plant genome should preferably be at least 20 consecutive nucleotides corresponding the native squalene epoxidase gene, and more preferably between 100 and the full DNA sequence of the gene. The homology of the added sequence may be at least 60%, and more preferably at least 70%, of the native plant gene.

B) Ribozyme Technology

Another method for downregulating gene expression by affecting mRNA levels is ribozyme technology. Ribozymes are RNA molecules capable of catalyzing the cleavage of RNA and other nucleic acids. In nature, Tetrahymena preribosomal RNA, some viroids, virusoids and satellites RNAs of plant viruses perform self-cleavage reactions. The cleavage site for some plant pathogenic RNAs consists of a consensus structure, called the "hammmerhead" motif. The cleavage occurs within this hammerhead 3' to a GUX

triplet, where X can be C, U, or A. The nucleotide region directing the catalysis of the cleavage reaction can be separated from the region where the cleavage occurs and the recognition of the target RNA can be modified by changing the nucleotide sequence of the regions flanking the cleavage site. As a consequence, ribozymes can be designed to catalyze cleavage reactions on targeted sequences of separate RNA substrates. This provides a means of regulating gene expression, if the DNA sequence of the gene is known.

In order to genetically engineer the down-regulation of a particular gene in plants, a vector can be constructed for transformation that includes one or more units, each of which may include a transcriptional promoter and a sequence encoding a ribozyme designed to cleave RNA transcribed from the gene or genes of interest. An example of this in plants has been provided by Schreier and co-workers (Steinecke et al. 1992, Wegener et al. 1994) in which a ribozyme was designed against neomycin phosphotransferase mRNA. Separate DNA constructs encoding the ribozyme and the neomycin phosphotransferase (npt) gene were used to transform plants. In plants containing both constructs, a reduction neomycin phosphotransferase activity was observed relative to plants transformed with only the npt gene construct.

Ribozyme technology also appears to be successful in other eukaryotes, such as the fruit fly (Zhao and Pick, 1993).

C) Co-suppression or Homology-Dependent Gene Silencing

When attempts have been made to overexpress homologous genes in plants, often a small fraction of the resulting transgenic plants are found to have very low levels of expression of both the native gene and the

introduced gene (transgene). This phenomenon has been called co-suppression or homology-dependent gene silencing (Stam et al. 1996, Matzke and Matzke 1995). The mechanism by which co-suppression occurs is very poorly understood. However, advantage can be taken of the phenomenon to down-regulate the expression of a gene of interest. This can be accomplished by transforming a plant with a DNA construct which contains a strong transcriptional promoter driving the sense transcription of a DNA sequence with high similarity to the gene of interest. For example, when the chalcone synthase gene was introduced into petunia in an attempt to overproduce chalcone synthase (which is involved in flower pigment biosynthesis), some transgenic plants showed pigment patterns and enzyme levels that indicated the suppression of chalcone synthase gene expression (Jorgensen 1990). Investigation of examples such as these has shown that the effect is often associated with repetition of the transgene inserts in the plant genome. Cosuppression may be dependent on the coding region of a gene or on the promoter and other non-coding regions.

Thus, the down-regulation of squalene epoxidase in plants may be engineered with the use of cDNA sequence that are disclosed herein, or with plant genomic sequences which may include the promoter or promoters of squalene epoxidase genes.

D) Other variations

Variations on the process of increasing squalene in plants include the use of different promoter sequences which may give rise to increased squalene in other tissues and at various stages of development. For example, the use of the cauliflower mosaic virus 35S promoter is likely to have an effect in most plant

tissues. Other seed-specific and tissue-specific promoter may also be used.

Also, other plant transformation methods may be used such as the particle gun technique (Christou 1993).

As well, other vectors, selectable markers, transcription terminators, etc., may be used (Guerineau and Mullineaux 1993).

It has already been observed that overexpression of a fragment of the hamster 3-hydroxymethyl-3-glutaryl CoA reductase (HMGR) gene in plants can elevate squalene levels in plants (Chappell et al. 1994). This is likely due to the fact that the level of HMGR limits the flow of carbon through the mevalonate/sterol pathway that includes squalene. It would be expected that a combination of elevated HMGR levels and down-regulated squalene epoxidase levels would have an effect on raising squalene levels that would be larger than the effect of either elevated HMGR alone or down-regulated squalene epoxidase alone.

## Experimental Detail

### IDENTIFICATION OF THE SQUALENE EPOXIDASE GENE

The DNA sequence of the squalene epoxidase gene of yeast was published by Jandrositz et al. (1991). Using the TBLASTN™ computer search program (Altschul et al. 1990) and the yeast squalene epoxidase (predicted) amino acid sequence, the sequence was used to search a database which included partial cDNA sequences called "the Non-Redundant database" maintained by the National Center for Biotechnology Information (NCBI) in the United States. This database is a non-redundant nucleotide database made up of:

| | |
|---|---|
| pdb | Brookhaven Protein Data Bank, April 1994 Release |
| genbank | Genbank® Release 87.0, February 15, 1995 |

| | |
|---|---|
| gbupdate | Genbank® cumulative updates to genbank major release |
| embl | EMBL data library, Release 41.0, December 1994 |
| emblu E | MBL Data Library, cumulative updates to embl major release |

maintained by the National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institute of Health, Bethesda, MD 20894, U.S.A.).

The database included expressed sequence tags (ESTs), i.e. partial sequences of more-or-less randomly chosen cDNA clones. This search identified the *Arabidopsis thaliana* cDNA clone 129F12T7 (Genbank accession no. T44667) as a putative squalene epoxidase gene. This clone was the seventh highest scoring sequence in this search and the highest scoring plant sequence. The P(N) of $1.9 \times 10^{-5}$ was considered borderline significant. The single high-scoring pair (HSP) of subsequences found was a stretch of 46 nucleotides with 21 positions identical (45%). Searches with the T44667 sequence revealed that a large portion of the 46 nucleotide region (29 nucleotides) matches a sequence motif found in a variety of enzymes that bound adenine dinucleotides, such as flavin adenine dinucleotide (FAD; which at least some squalene epoxidases are known to use as a cofactor; see Wierenga et al. 1986). So, in fact, the search, done when only the partial DNA sequence (T44667) was available, suggested the possibility, but did not confirm that T44667 corresponded to a squalene epoxidase gene.

The 129F12T7 clone was obtained and its DNA sequenced completely by the inventors at the Plant Biotech Institute of the National Research Council of Canada at Saskatoon, Saskatchewan, Canada. The DNA sequence of the cDNA insert of p129F12T7 is shown in the Sequence Listing (see later) as SEQ ID NO: 1. After the full sequence of the insert of p129F12T7 was obtained,

the Non-Redundant Protein Database (NCBI) was searched using the BLAST™ software (Altschul et al. 1990) (NCBI) based on the predicted amino acid sequence. The amino acid sequence corresponding to the open reading frame of SEQ ID NO:1 are shown in the Sequence Listing as SEQ ID NO:2. The *Arabidopsis* sequence gave the highest scoring matches with squalene epoxidase sequences including that of rat ($P(N)=5 \times 10^{-60}$) and yeast ($P(N)=9.2 \times 10^{-33}$). No sequences which had been reliably identified had P(N) values less than $10^{-6}$. These numbers indicate that the product of the *Arabidopsis* gene is, in all probability, squalene epoxidase.

The 129F12T7 clone was used to probe a *B. napus* cDNA library, obtained from Dr. Edward Tsang of the Plant Biotech Institute. Two independent clones, pDR111 and pDR411 were isolated and sequenced. The Sequence Listing shows the DNA sequences of the cDNA inserts of pDR111 [SEQ ID NO:3] and pDR411 [SEQ ID NO:5] and the amino acid sequences corresponding to the coding regions of SEQ ID NO:3 [SEQ ID NO:4] and SEQ ID NO:5 [SEQ ID NO:11]. pDR111 and pDR411 have similar (but not identical) DNA sequences which are also similar to the 129F12T7 sequence. Plasmids p129F12T7, pDR111 and pDR411 were deposited at the American Type Culture Collection (ATCC), 12901 Parklawn Drive, Rockville, Maryland 20852-1776, USA, under the terms of the Budapest Treaty on January 9, 1997 and were accepted. The deposit numbers are, respectively, ATCC 97847, ATCC 97846 and ATCC 97845. A single deposit receipt and statement of viability was issued for all three deposits on January 17, 1997.

Figure 1 of the accompanying drawings shows an alignment of amino acid sequences for the 129F12T7 clone [SEQ ID NO:2], the pDR111 clone [SEQ ID NO:4] and the pDR411 [SEQ ID NO:11] clone, along with the squalene epoxidase sequences amino acid sequences for mouse [SEQ ID NO:6], rat [SEQ ID NO:7] and yeast [SEQ ID NO:8]. The

plant sequence show blocks of high similarity to the non-plant sequences, including the region thought to correspond to an adenine dinucleotide-binding site (residues 45-88 of the *Arabidopsis* sequence; Wierenga et al. 1986; Sakakibara et al. 1995), as well as in the C-terminal half of the sequence. The amino acid sequence similarities based on this alignment are shown in Table 1 below.

Table 1

Amino acid sequence similarities calculated by MEGALIGN™ software for the sequence alignment of Figure 1.

| | PDR411 Predicted Amino Acid Sequence | p129F12T7 Predicted Amino Acid Sequence | Mouse Squalene Epoxidase Predicted Amino Acid Sequence | Rat Squalene Epoxidase Predicted Amino Acid Sequence | Yeast Squalene Epoxidase Predicted Amino Acid Sequence |
|---|---|---|---|---|---|
| pDR111 Predicted Amino Acid Sequence | 74.8 | 59.6 | 27.0 | 26.4 | 21.5 |
| pDR411 Predicted Amino Acid Sequence | | 62.9 | 29.2 | 27.8 | 21.3 |
| p129F12T7 Predicted Amino Acid Sequence | | | 27.3 | 26.1 | 20.9 |
| Mouse Squalene Epoxidase Predicted Amino Acid Sequence | | | | 91.8 | 30.4 |
| Rat Squalene Epoxidase Predicted Amino Acid Sequence | | | | | 30.4 |

Analysis of the pDR411 sequence suggests it has an intron in the 3'-end of its amino acid coding region which is, of course, unusual in cDNA. If nucleotides 1473-1629 (inclusive) are removed from the sequence and

the cDNA translated, the C-terminus is more similar to the pDR111 and p129F12T7 amino acid sequences [SEQ ID NO:4 and SEQ ID NO:2]. Also, there are sequence patterns in this region that are common to other plant introns (5' and 3' splice consensus sequences and high AT content (Goodall and Filipowicz, 1991)). This may mean that the pDR411 clone represents an intermediate or precursor RNA, rather than the final messenger RNA (mRNA). There can therefore be less certainty in predicting the full amino acid sequence corresponding to pDR411, although this predicted sequence is shown in Fig. 1 [SEQ ID NO:11]. However, the possible presence of a small intron in the 3'-end of pDR411 does not cause a problem for its use in antisense techniques.

Employing the plant squalene epoxidase sequences, transgenic plants can be generated which accumulate squalene in their seeds. This can be done by established genetic transformation methods using DNA constructs that include the napin or other seed-specific promoters (Kridl, 1988; Anonymous, 1995) and fragments of plant squalene epoxidase genes arranged in the antisense orientation. Downregulation of the squalene epoxidase gene in seeds by antisense technology (Inouye, 1990; Bourque, 1995) will prevent the conversion of squalene to squalene expoxide and result in squalene accumulation.

<u>ISOLATION OF SQUALENE EPOXIDASE GENE IN *B. NAPUS*</u>

The 129F12T7 clone obtained as described above was used to probe for the homologous gene in *B. napus* as follows.

Unless otherwise noted all molecular biology methods were performed as described in Ausubel et al.(1994).

The *Arabidopsis* 129F12T7 DNA Probe

The plasmid p129F12T7 was digested with the restriction enzymes Sal I and Not I. The resulting DNA

fragments were separated by agarose gel electrophoresis. The 1.8kb Sal I/Not I DNA fragment corresponding to the *Arabidopsis* squalene epoxidase cDNA was purified from a gel band. A radiolabelled DNA probe was prepared by the random priming method and [alpha-32P]-dCTP (deoxycytidine triphosphate).

Library Screening

The probe produced as above was used to screen a *B. napus* cDNA library, kindly provided by Dr. Edward Tsang of the Plant Biotechnology Institute (Saskatoon, Saskatchewan, Canada). To construct the library, *B. napus* seedlings (cv. Westar) were grown (on half strength Murashige and Skoog agar (1%) medium supplemented with 1% sucrose) in the dark at 22°C for two weeks after germination and exposed to light for 24 hours. PolyA+ RNA was extracted from the seedlings and first strand cDNA synthesis was primed with an oligo dT/Not I adapter/primer. Sal I adapters were ligated after second strand cDNA synthesis and a library was constructed in Not I/Sal I arms of the LambdaZipLox vector (Life Technologies).

The library was plated using standard methods and the Y1090 strain of *E. coli*. Approximately 25,000 plaques from the library were plated, lifted onto Hybond®-C nylon membranes (Amersham)and hybridized with the above probe according to the manufacturer's instructions. After two rounds of plaque purification, two independent clones, pDR111 and pDR411 were isolated by *in vivo* excision.

The p129F12T7, pDR111 and pDR411 clones were sequenced using the PRISM® DyeDeoxy Terminator Cycle Sequencing System (Perkin Elmer/Applied Biosystems) and a

Model 373 DNA Sequencer (Applied Biosystems). DNA sequences were assembled and analyzed using the Lasergene® suite of software (DNASTAR, Inc.) and BLAST® and related software of the NCBI.

CONSTRUCTION OF VECTORS FOR PLANT TRANSFORMATION

Figs. 2, 3 and 4 show three vectors constructed for plant transformation, namely pSE129A, pSE111A and pSE411A. In these drawings, the following abbreviations are used:

| | |
|---|---|
| nosT | 3'-terminus of the nopaline synthase gene |
| SE129 | Sal I/Not I insert of p129F12T7 |
| SE111 | Sal I/Xba I fragment of the insert of pDR111 |
| SE411 | Sal I/Not I insert of pDR411 |
| Napin P | napin gene promoter (Josefsson 1986). |

All other elements are described by Guerineau and Mullineaux (1993), Thomas et al. (1992) and Beban (1984).

These plasmids were constructed as follows.

pDH1

The plasmid pE35SNT was obtained from Raju Datla (Plant Biotechnology Institute, Saskatoon, Saskatchewan Canada). It contains a double 35S promoter and nopaline synthase (Nos) terminator (Datla, 1992) in pUC19. It was digested with Hind III and Xba I to remove the double 35S promoter. The napin promoter (Josefsson et al. 1987) was isolated from pNap (obtained from Ravi Jain, Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada) by Hind III and Xba I digestion. The plasmid pDH1 was produced by ligation of the large pE35SNT/Hind III/Xba I fragment and the Hind III/Xba I napin promoter fragment. Thus, pDH1 contained the napin promoter and the Nos

terminator between the Hind III and EcoR I sites of the pUC19 vector.

pSE129A

The p129F12T7 plasmid was digested with Pst I and Hind III. The fragment containing the *Arabidopsis* squalene epoxidase cDNA was ligated to the Pst I- and Hind III-digested vector pTrcHisB (INVITROGEN®) to give the circular plasmid pTrcHis129. pTrcHis129 was digested with Xba I and BamH I and the squalene epoxidase cDNA fragment was ligated into Xba I- and BamH I-digested pDH1. The resulting plasmid pDH129A contained the squalene epoxidase cDNA in antisense orientation downstream from the napin promoter and upstream of the Nos terminator. pDH129A was digested with Hind III and partially digested EcoR I and the fragment containing napin promoter, squalene epoxidase cDNA and Nos terminator was ligated into Hind III- and EcoR I-digested pRD400 (a binary vector for plant transformation containing a gene conferring kanamycin resistance; (Datla et al. 1992)) to give pSE129A.

pSE111A

The pDR111 plasmid was digested with Sma I and Xba I. The fragment containing a *B. napus* squalene epoxidase cDNA (excluding a small part of the 3' end downstream of the Xba I site) was ligated to the large fragment of Sma I- and XBa I-digested pDH129 vector (containing the napin promoter and Nos terminator) to give the circular plasmid pDH111A. pDH111A contained the squalene epoxidase cDNA in antisense orientation downstream from the napin promoter and upstream of the Nos terminator. pDH111A was digested with Hind III and partially with EcoR I and the fragment containing napin promoter, cDNA and Nos terminator was ligated into Hind III- and EcoR I-digested pRD400 to give pSE111A.

pSE411A

The pDR411 plasmid was digested with Sma I and Xba I. The fragment containing a *B. napus* squalene epoxidase cDNA was ligated to the large fragment of Sma I- and Xba I-digested pDH129A vector (containing the napin promoter and Nos terminator and excluding the *Arabidopsis* cDNA sequence) to give the circular plasmid pDH411A. pDH411A contained the squalene epoxidase cDNA in antisense orientation downstream from the napin promoter and upstream of the Nos terminator. pDH111A was digested with EcoR I and partially digested with Hind III and the fragment containing napin promoter, squalene epoxidase cDNA and Nos terminator was ligated into Hind III- and EcoR I-digested pRD400 (Datla et al. 1992) to give pSE411A.

The final vectors pSE129A, pSE111A and pSE411A were deposited on March 5, 1997 under the terms of the Budapest Treaty at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA; under deposit nos. ATCC 97910, ATCC 97909 and ATCC 97908, respectively). These vectors were introduced into *Agrobacterium tumefaciens* strain GV3101 (bearing helper plasmid pMP90; Koncz and Schell, 1986) by electroporation.

PLANT GROWTH CONDITIONS

All *A. thaliana* control and transgenic plants were grown in controlled growth chambers, under continuous fluorescent illumination (150-200 $\mu E \cdot m^{-2}\ sec^{-1}$) at 22°C, as described by Katavic et al.(1995).

PLANT TRANSFORMATION

The pSE129A construct was tested in *A. thaliana* by *in planta* transformation techniques.

Wild type (WT) *A. thaliana* plants of ecotype Columbia were grown in soil. *In planta* transformation was performed by vacuum infiltration (Bechtold et al. 1993) with overnight bacterial suspension of *A. tumefaciens* strain GV3101 bearing helper nopaline plasmid pMP90 (disarmed Ti plasmid with intact *vir* region acting *in trans*, gentamycin and kanamycin selection markers; Koncz and Schell (1986)) and binary vector pSE129A.

After infiltration, plants were grown to set seeds ($T_1$ generation). Dry seeds ($T_1$ generation of seeds) were harvested in bulk and screened on selective medium with 50 mg/L kanamycin. After two to three weeks on selective medium, surviving seedlings were transferred to soil. Mature seeds from these seedlings ($T_2$ seeds) were used for squalene analysis. Mature seeds from untransformed wild type (WT) Columbia plants and pRD400 transgenic plants (binary vector pRD400, containing only kanamycin selection marker; Datla et al. 1992) were used as controls in analyses of seed lipids.

Seed Analysis

Seeds were analyzed for squalene levels as follows:

In all steps, care was taken to avoid contamination from external sources, particularly human skin. 5-10mg of *Arabidopsis* seeds were weighed and rinsed with hexane to remove any external contamination. 1 ml of 7.5% KOH (in 95% methanol) was added to each sample and 250ng of squalane were added as internal standard. (Squalane is the hydrogenated form of squalene.) Seeds were homogenized with a Polytron® (Model PRO200, PRO Scientific) at maximum speed for 40 seconds. The head of

the Polytron was washed with 1 ml of 7.5% KOH (in 95% methanol) and the wash was pooled with the homogenate. The mixture was incubated at 80°C for 1 hr, then cooled to room temperature. The mixture was centrifuged at 3000 g for 5 min, and the supernatant was transferred to a fresh tube. One ml of $H_2O$ and 1.5 ml of hexane were added to the supernatant and, after vortexing, the mixture was centrifuged at 3000 *g* for 5 minutes. The hexane (top) layer was transferred to another test tube. The aqueous phase was re-extracted with 1.5 ml hexane and the hexane fractions were pooled. The hexane fraction was extracted with 1 ml of water/methanol/KOH (50:50:2) and evaporated under nitrogen. The residue was dissolved in 50 ul of hexane and transferred to an autosampler vial. Gas-liquid chromatography was performed with a DB5 column (J & W Scientific, USA) using the following parameters:

| | | |
|---|---|---|
| Column Temperature : | 0-1 min | 180°C |
| | 1-16 min | 180-280°C (linear ramp) |
| | 16-30 min | 280°C |
| Injector Temperature | | 275°C |
| Detector Temperature | | 300°C. |

Transgenic Results

Seeds from 9 *Arabidopsis* lines transformed with pRD400 and 55 lines transformed with pSE129A were analyzed for squalene content. Table 2 below shows the results for all of the pRD400 transgenic lines and 4 pSE129A lines.

<u>Table 2</u>

| Line | Vector | Squalene ug/g dry weight | Standard Deviation of 3 Assays |
|---|---|---|---|
| k401 | pRD400 | 4.04 | 0.5 |
| k402 | pRD400 | 4.71 | 0.16 |
| k403 | pRD400 | 4.39 | 0.34 |
| k404 | pRD400 | 4.86 | 0.75 |
| k405 | pRD400 | 3.92 | 0.92 |
| k406 | pRD400 | 4.04 | 1.68 |
| k409 | pRD400 | 5.03 | 0.85 |
| k410 | pRD400 | 6.09 | 1.22 |
| k411 | pRD400 | 4.57 | 1.26 |
| | | | |
| k9 | pSE129A | 9.96 | 1.59 |
| k12 | pSE129A | 11.34 | 2.01 |
| k50 | pSE129A | 12.38 | 0.35 |
| k54 | pSE129A | 9.76 | 1.43 |

The mean and standard deviation of the 9 pRD400 lines is 4.6 and 0.7, respectively.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: National Research Council of Canada
(B) STREET: 1200 Montreal Road
(C) CITY: Ottawa
(D) STATE: Ontario
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(D) STATE: SK
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 3A9
(G) TELEPHONE: (306) 975-5269
(H) TELEFAX: (306) 975-4839

(A) NAME: Dr. Martin J.T. Reaney
(B) STREET: 1027 5th Street East
(C) CITY: Saskatoon
(D) STATE: SK
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 1H3

(A) NAME: Dr. Samuel L. MacKenzie
(B) STREET: 17 Cambridge Crescent
(C) CITY: Saskatoon
(D) STATE: SK
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): S7H 3P9

(ii) TITLE OF INVENTION: Process for Raising Squalene Levels in Plants and DNA Sequences Used Therefor

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1756 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: Columbia

(D) DEVELOPMENTAL STAGE: 3 different stages

(F) TISSUE TYPE: 4 different tissues

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Lambda-PRL2

(B) CLONE: 129F12T7

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:15..1565

(D) OTHER INFORMATION:/codon_start= 15

/function= "converts squalene to

2,3-oxidosqualene"

/EC_number= 1.14.99.7
/product= "squalene epoxidase"
/standard_name= "squalene monooxygenase (2,3-epoxidizing)"

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION:1566..1756

(ix) FEATURE:
(A) NAME/KEY: polyA_site
(B) LOCATION:1756

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION:1..14

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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                Met Thr Tyr Ala Trp Leu Trp Thr Leu Leu Ala Phe
                  1               5                  10

GTT CTG ACA TGG ATG GTT TTT CAC CTC ATC AAG ATG AAG AAG GCG GCA        98
Val Leu Thr Trp Met Val Phe His Leu Ile Lys Met Lys Lys Ala Ala
         15                  20                  25

ACC GGA GAT TTA GAG GCC GAG GCA GAA GCA AGA AGA GAT GGT GCA ACG       146
Thr Gly Asp Leu Glu Ala Glu Ala Glu Ala Arg Arg Asp Gly Ala Thr
     30                  35                  40

GAT GTC ATC ATT GTT GGG GCG GGT GTT GCA GGC GCT TCT CTT GCT TAT       194
Asp Val Ile Ile Val Gly Ala Gly Val Ala Gly Ala Ser Leu Ala Tyr
 45                  50                  55                  60

GCT TTA GCT AAG GAT GGA CGA CGA GTA CAT GTG ATA GAG AGG GAC TTA       242
Ala Leu Ala Lys Asp Gly Arg Arg Val His Val Ile Glu Arg Asp Leu
                 65                  70                  75
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Lys Glu Pro Gln Arg Phe Met Gly Glu Leu Met Gln Ala Gly Gly Arg
80 85 90

TTC ATG TTA GCC CAG CTT GGC CTC GAA GAT TGT TTG GAG GAC ATA GAC 338
Phe Met Leu Ala Gln Leu Gly Leu Glu Asp Cys Leu Glu Asp Ile Asp
95 100 105

GCA CAA GAA GCG AAG TCC TTG GCA ATA TAC AAG GAT GGA AAA CAC GCG 386
Ala Gln Glu Ala Lys Ser Leu Ala Ile Tyr Lys Asp Gly Lys His Ala
110 115 120

ACA TTG CCT TTT CCA GAT GAC AAG AGT TTT CCT CAT GAG CCA GTA GGT 434
Thr Leu Pro Phe Pro Asp Asp Lys Ser Phe Pro His Glu Pro Val Gly
125 130 135 140

AGA CTC TTA CGT AAT GGT CGG CTG GTA CAA CGT TTA CGC CAA AAA GCA 482
Arg Leu Leu Arg Asn Gly Arg Leu Val Gln Arg Leu Arg Gln Lys Ala
145 150 155

GCT TCT CTT AGC AAT GTT CAA TTA GAA GAA GGA ACA GTG AAG TCT TTA 530
Ala Ser Leu Ser Asn Val Gln Leu Glu Glu Gly Thr Val Lys Ser Leu
160 165 170

ATT GAA GAA GAA GGA GTG GTC AAA GGA GTG ACA TAC AAA AAT AGC GCA 578
Ile Glu Glu Glu Gly Val Val Lys Gly Val Thr Tyr Lys Asn Ser Ala
175 180 185

GGC GAA GAA ATA ACG GCC TTT GCA CCT CTT ACT GTC GTA TGC GAT GGT 626
Gly Glu Glu Ile Thr Ala Phe Ala Pro Leu Thr Val Val Cys Asp Gly
190 195 200

TGT TAT TCG AAC CTT CGT CGG TCA CTC GTG GAT AAT ACT GAG GAA GTC 674
Cys Tyr Ser Asn Leu Arg Arg Ser Leu Val Asp Asn Thr Glu Glu Val
205 210 215 220

CTC TCG TAC ATG GTG GGT TAC GTC ACG AAG AAT AGC CGA CTT GAA GAT 722
Leu Ser Tyr Met Val Gly Tyr Val Thr Lys Asn Ser Arg Leu Glu Asp
225 230 235

CCC CAT AGT CTA CAT TTG ATA TTT TCT AAA CCT TTG GTT TGT GTT ATA 770
Pro His Ser Leu His Leu Ile Phe Ser Lys Pro Leu Val Cys Val Ile
240 245 250

TAT CAA ATA ACC AGT GAT GAA GTT CGT TGT GTT GCC GAA GTT CCC GCT 818
Tyr Gln Ile Thr Ser Asp Glu Val Arg Cys Val Ala Glu Val Pro Ala
255 260 265

GAT AGT ATT CCT TCT ATA TCG AAT GGT GAA ATG TCT ACC TTC CTC AAG 866
Asp Ser Ile Pro Ser Ile Ser Asn Gly Glu Met Ser Thr Phe Leu Lys
270 275 280

AAA TCA ATG GCT CCT CAG ATA CCT GAA ACT GGA AAT CTT CGG GAG ATA 914
Lys Ser Met Ala Pro Gln Ile Pro Glu Thr Gly Asn Leu Arg Glu Ile
285 290 295 300

TTT TTG AAA GGC ATA GAG GAA GGA TTA CCA GAG ATA AAA TCA ACA GCG 962
Phe Leu Lys Gly Ile Glu Glu Gly Leu Pro Glu Ile Lys Ser Thr Ala
305 310 315

ACG AAA AGT ATG TCA TCG AGA TTG TGT GAT AAA AGA GGA GTG ATT GTG 1010
Thr Lys Ser Met Ser Ser Arg Leu Cys Asp Lys Arg Gly Val Ile Val
320 325 330

TTG GGA GAT GCA TTC AAT ATG CGT CAT CCT ATA ATC GCG TCA GGA ATG 1058
Leu Gly Asp Ala Phe Asn Met Arg His Pro Ile Ile Ala Ser Gly Met
335 340 345

ATG GTT GCA CTC TCG GAC ATT TGC ATT CTA CGC AAT CTT CTC AAA CCA 1106
Met Val Ala Leu Ser Asp Ile Cys Ile Leu Arg Asn Leu Leu Lys Pro
350 355 360

TTG CCT AAC CTC AGC AAT ACT AAG AAA GTC TCT GAT CTT GTC AAG TCC 1154
Leu Pro Asn Leu Ser Asn Thr Lys Lys Val Ser Asp Leu Val Lys Ser
365 370 375 380

TTT TAC ATC ATC CGC AAG CCA ATG TCA GCG ACC GTG AAC ACG CTC GCG 1202
Phe Tyr Ile Ile Arg Lys Pro Met Ser Ala Thr Val Asn Thr Leu Ala
385 390 395

AGT ATC TTT TCA CAA GTG CTT GTT GCT ACA ACA GAC GAA GCA AGA GAG 1250
Ser Ile Phe Ser Gln Val Leu Val Ala Thr Thr Asp Glu Ala Arg Glu
400 405 410

GGA ATG CGA CAA GGC TGC TTC AAT TAC CTA GCT CGT GGA GAT TTT AAA 1298
Gly Met Arg Gln Gly Cys Phe Asn Tyr Leu Ala Arg Gly Asp Phe Lys
415 420 425

ACA AGG GGA TTG ATG ACT ATT CTC GGA GGC ATG AAC CCT CAC CCT CTT 1346
Thr Arg Gly Leu Met Thr Ile Leu Gly Gly Met Asn Pro His Pro Leu
430 435 440

ACT CTA GTC CTT CAT CTT GTA GCC ATC ACC CTT ACG TCC ATG GGC CAC 1394
Thr Leu Val Leu His Leu Val Ala Ile Thr Leu Thr Ser Met Gly His
445 450 455 460

TTG CTC TCT CCG TTT CCT TCG CCT CGT CGC TTT TGG CAT AGC CTC AGA 1442
Leu Leu Ser Pro Phe Pro Ser Pro Arg Arg Phe Trp His Ser Leu Arg
465 470 475

ATT CTT GCC TGG GCT TTG CAA ATG TTG GGT GCA CAT TTA GTG GAT GAA 1490
Ile Leu Ala Trp Ala Leu Gln Met Leu Gly Ala His Leu Val Asp Glu
480 485 490

GGA TTC AAG GAA ATG TTG ATT CCA ACA AAC GCA GCT GCT TAT CGA AGG 1538
Gly Phe Lys Glu Met Leu Ile Pro Thr Asn Ala Ala Ala Tyr Arg Arg
495 500 505

AAC TAT ATC GCC ACA ACC ACT GTT TGA TCAATCCATA ACACGAAGAC 1585
Asn Tyr Ile Ala Thr Thr Thr Val
510 515

TGTTTTATTC GGAGATGAAA AATAACAACT CAAACAGTTA ACTTTCTACA ACCAAATAAA 1645

TAATTGTGTG TATATGAAGT TGAGCCTATG GTTAAGCTCT ACTGAATTGT GTTGAAAACA 1705

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 516 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Thr Tyr Ala Trp Leu Trp Thr Leu Leu Ala Phe Val Leu Thr Trp
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Met Val Phe His Leu Ile Lys Met Lys Lys Ala Ala Thr Gly Asp Leu
20 25 30

Glu Ala Glu Ala Glu Ala Arg Arg Asp Gly Ala Thr Asp Val Ile Ile
35 40 45

Val Gly Ala Gly Val Ala Gly Ala Ser Leu Ala Tyr Ala Leu Ala Lys
50 55 60

Asp Gly Arg Arg Val His Val Ile Glu Arg Asp Leu Lys Glu Pro Gln
65 70 75 80

Arg Phe Met Gly Glu Leu Met Gln Ala Gly Gly Arg Phe Met Leu Ala
85 90 95

Gln Leu Gly Leu Glu Asp Cys Leu Glu Asp Ile Asp Ala Gln Glu Ala
100 105 110

Lys Ser Leu Ala Ile Tyr Lys Asp Gly Lys His Ala Thr Leu Pro Phe
115 120 125

Pro Asp Asp Lys Ser Phe Pro His Glu Pro Val Gly Arg Leu Leu Arg
130 135 140

Asn Gly Arg Leu Val Gln Arg Leu Arg Gln Lys Ala Ala Ser Leu Ser
145 150 155 160

Asn Val Gln Leu Glu Glu Gly Thr Val Lys Ser Leu Ile Glu Glu Glu

165 170 175

Gly Val Val Lys Gly Val Thr Tyr Lys Asn Ser Ala Gly Glu Glu Ile
180 185 190

Thr Ala Phe Ala Pro Leu Thr Val Val Cys Asp Gly Cys Tyr Ser Asn
195 200 205

Leu Arg Arg Ser Leu Val Asp Asn Thr Glu Glu Val Leu Ser Tyr Met
210 215 220

Val Gly Tyr Val Thr Lys Asn Ser Arg Leu Glu Asp Pro His Ser Leu
225 230 235 240

His Leu Ile Phe Ser Lys Pro Leu Val Cys Val Ile Tyr Gln Ile Thr
245 250 255

Ser Asp Glu Val Arg Cys Val Ala Glu Val Pro Ala Asp Ser Ile Pro
260 265 270

Ser Ile Ser Asn Gly Glu Met Ser Thr Phe Leu Lys Lys Ser Met Ala
275 280 285

Pro Gln Ile Pro Glu Thr Gly Asn Leu Arg Glu Ile Phe Leu Lys Gly
290 295 300

Ile Glu Glu Gly Leu Pro Glu Ile Lys Ser Thr Ala Thr Lys Ser Met
305 310 315 320

Ser Ser Arg Leu Cys Asp Lys Arg Gly Val Ile Val Leu Gly Asp Ala
325 330 335

Phe Asn Met Arg His Pro Ile Ile Ala Ser Gly Met Met Val Ala Leu
340 345 350

Ser Asp Ile Cys Ile Leu Arg Asn Leu Leu Lys Pro Leu Pro Asn Leu
355 360 365

Ser Asn Thr Lys Lys Val Ser Asp Leu Val Lys Ser Phe Tyr Ile Ile
370 375 380

Arg Lys Pro Met Ser Ala Thr Val Asn Thr Leu Ala Ser Ile Phe Ser
385 390 395 400

Gln Val Leu Val Ala Thr Thr Asp Glu Ala Arg Glu Gly Met Arg Gln
405 410 415

Gly Cys Phe Asn Tyr Leu Ala Arg Gly Asp Phe Lys Thr Arg Gly Leu
420 425 430

Met Thr Ile Leu Gly Gly Met Asn Pro His Pro Leu Thr Leu Val Leu
435 440 445

His Leu Val Ala Ile Thr Leu Thr Ser Met Gly His Leu Leu Ser Pro
450 455 460

Phe Pro Ser Pro Arg Arg Phe Trp His Ser Leu Arg Ile Leu Ala Trp
465 470 475 480

Ala Leu Gln Met Leu Gly Ala His Leu Val Asp Glu Gly Phe Lys Glu
485 490 495

Met Leu Ile Pro Thr Asn Ala Ala Ala Tyr Arg Arg Asn Tyr Ile Ala
500 505 510

Thr Thr Thr Val
515

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1748 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Brassica napus

(B) STRAIN: Westar

(D) DEVELOPMENTAL STAGE: 14 day greening-etiolated

(F) TISSUE TYPE: hypocotyls

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Tsang

(B) CLONE: pDR111

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..18

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:19..1575

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:1576..1748

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CCACGCGTCC GAAAAGAT ATG GAT ATG GCT TTT GTG GAA GTT TGT TTA CGG      51
                    Met Asp Met Ala Phe Val Glu Val Cys Leu Arg
                            520                 525

ATG CTA CTT GTC TTC GTA CTG TCT TGG ACG ATA TTT CAC GTC AAC AAC      99
Met Leu Leu Val Phe Val Leu Ser Trp Thr Ile Phe His Val Asn Asn
    530                 535                 540

AGG AAG AAG AAG AAG GCG ACG AAG TTG GCG GAT CTG GCT ACT GAG GAG     147
Arg Lys Lys Lys Lys Ala Thr Lys Leu Ala Asp Leu Ala Thr Glu Glu
545                 550                 555                 560
```

AGA AAA GAA GGT GGC CCT GAC GTC ATA ATA GTC GGA GCT GGA GTG GGC 195
Arg Lys Glu Gly Gly Pro Asp Val Ile Ile Val Gly Ala Gly Val Gly
565 570 575

GGC TCA GCT CTC GCC TAT GCT CTT GCT AAG GAC GGG CGT CGA GTA CAT 243
Gly Ser Ala Leu Ala Tyr Ala Leu Ala Lys Asp Gly Arg Arg Val His
580 585 590

GTG ATA GAA AGA GAC ATG AGA GAG CCA GTG AGA ATG ATG GGT GAG TTC 291
Val Ile Glu Arg Asp Met Arg Glu Pro Val Arg Met Met Gly Glu Phe
595 600 605

ATG CAG CCA GGA GGA CGG CTC ATG CTT TCT AAG CTC GGT CTT CAA GAT 339
Met Gln Pro Gly Gly Arg Leu Met Leu Ser Lys Leu Gly Leu Gln Asp
610 615 620

TGT TTA GAG GAA ATA GAC GCA CAG AAA TCC ACC GGC ATA AGA CTT TTT 387
Cys Leu Glu Glu Ile Asp Ala Gln Lys Ser Thr Gly Ile Arg Leu Phe
625 630 635 640

AAG GAC GGA AAA GAA ACT GTC GCA TGT TTT CCG GTG GAC ACC AAC TTT 435
Lys Asp Gly Lys Glu Thr Val Ala Cys Phe Pro Val Asp Thr Asn Phe
645 650 655

CCT TAT GAA CCA TCT GGT CGA TTT TTT CAC AAT GGC CGT TTT GTC CAG 483
Pro Tyr Glu Pro Ser Gly Arg Phe Phe His Asn Gly Arg Phe Val Gln
660 665 670

AGA CTG CGC CAA AAG GCC TCT TCT CTT CCC AAT GTG CGG CTG GAA GAA 531
Arg Leu Arg Gln Lys Ala Ser Ser Leu Pro Asn Val Arg Leu Glu Glu
675 680 685

GGG ACC GTC CGA TCT TTG ATA GAA GAA AAA GGA GTG GTC AAA GGA GTG 579
Gly Thr Val Arg Ser Leu Ile Glu Glu Lys Gly Val Val Lys Gly Val
690 695 700

ACA TAC AAG AAC AGT TCA GGG GAA GAA ACC ACA TCA TTT GCA CCT CTC 627
Thr Tyr Lys Asn Ser Ser Gly Glu Glu Thr Thr Ser Phe Ala Pro Leu
705 710 715 720

ACT GTC GTA TGC GAT GGT TGC CAC TCG AAC CTT CGT CGC TCT CTA AAT 675
Thr Val Val Cys Asp Gly Cys His Ser Asn Leu Arg Arg Ser Leu Asn
725 730 735

GAC AAC AAT GCG GAG GTT ACG GCG TAC GAG ATT GGT TAC ATC TCG AGG 723
Asp Asn Asn Ala Glu Val Thr Ala Tyr Glu Ile Gly Tyr Ile Ser Arg
740 745 750

AAT TGT CGC CTT GAA CAG CCC GAC AAG TTA CAC TTG ATA ATG GCT AAA 771
Asn Cys Arg Leu Glu Gln Pro Asp Lys Leu His Leu Ile Met Ala Lys
755 760 765

CCG TCT TTC GCC ATG TTG TAT CAA GTC AGC AGC ACC GAC GTT CGT TGT 819
Pro Ser Phe Ala Met Leu Tyr Gln Val Ser Ser Thr Asp Val Arg Cys
770 775 780

AAT TTT GAG CTT CTC TCC AAA AAT CTT CCT TCT GTT TCA AAT GGT GAA 867
Asn Phe Glu Leu Leu Ser Lys Asn Leu Pro Ser Val Ser Asn Gly Glu
785 790 795 800

ATG ACG TCC TTC GTG AGG AAC TCT ATT GCT CCC CAG GTA CCT CTA AAA 915
Met Thr Ser Phe Val Arg Asn Ser Ile Ala Pro Gln Val Pro Leu Lys
805 810 815

CTC CGC AAA ACA TTT TTG AAA GGG CTC GAT GAG GGA TCA CAT ATA AAA 963
Leu Arg Lys Thr Phe Leu Lys Gly Leu Asp Glu Gly Ser His Ile Lys
820 825 830

ATT ACA CAA GCA AAG CGC ATC CCA GCT ACT TTG AGC AGA AAA AAG GGA 1011
Ile Thr Gln Ala Lys Arg Ile Pro Ala Thr Leu Ser Arg Lys Lys Gly
835 840 845

GTG ATT GTG TTG GGA GAT GCA TTC AAC ATG CGT CAT CCC GTA ATC GCG 1059
Val Ile Val Leu Gly Asp Ala Phe Asn Met Arg His Pro Val Ile Ala
850 855 860

TCG GGG ATG ATG GTT TTA TTG TCT GAC ATT CTC ATT CTA AGC CGT CTT 1107
Ser Gly Met Met Val Leu Leu Ser Asp Ile Leu Ile Leu Ser Arg Leu
865 870 875 880

CTC AAG CCT TTG GGC AAC CTC GGT GAT GAA AAC AAA GTC TCA GAA GTT 1155
Leu Lys Pro Leu Gly Asn Leu Gly Asp Glu Asn Lys Val Ser Glu Val
885 890 895

ATG AAG TCC TTC TAT GCT CTA CGC AAG CCA ATG TCA GCA ACA GTA AAC 1203
Met Lys Ser Phe Tyr Ala Leu Arg Lys Pro Met Ser Ala Thr Val Asn
900 905 910

ACA CTA GGG AAT TCA TTT TGG CAA GTG CTA ATT GCT TCA ACG GAC GAA 1251
Thr Leu Gly Asn Ser Phe Trp Gln Val Leu Ile Ala Ser Thr Asp Glu
915 920 925

GCA AAA GAG GCC ATG CGA CAA GGT TGC TTT GAT TAC CTC TCT AGT GGT 1299
Ala Lys Glu Ala Met Arg Gln Gly Cys Phe Asp Tyr Leu Ser Ser Gly
930 935 940

GGG TTT CGC ACG TCA GGC TTG ATG GCT CTG ATT GGT GGC ATG AAC CCT 1347
Gly Phe Arg Thr Ser Gly Leu Met Ala Leu Ile Gly Gly Met Asn Pro
945 950 955 960

AGG CCA CTT TCT CTC TTC TAT CAT CTA TTC GTT ATT TCT TTA TCC TCC 1395
Arg Pro Leu Ser Leu Phe Tyr His Leu Phe Val Ile Ser Leu Ser Ser
965 970 975

ATT GGC CAA CTG CTC TCT CCA TTC CCC ACT CCT CTT CGT GTT TGG CAT 1443
Ile Gly Gln Leu Leu Ser Pro Phe Pro Thr Pro Leu Arg Val Trp His
980 985 990

AGC CTC AGA CTT CTT GAT TTG TCT TTG AAA ATG TTG GTT CCT CAT CTC 1491
Ser Leu Arg Leu Leu Asp Leu Ser Leu Lys Met Leu Val Pro His Leu
995 1000 1005

AAG GCC GAA GGA ATA GGT CAA ATG TTG TCT CCA ACA AAT GCA GCG GCG 1539
Lys Ala Glu Gly Ile Gly Gln Met Leu Ser Pro Thr Asn Ala Ala Ala
1010 1015 1020

TAT CGC AAA AGC TAT ATG GCT GCA ACC GTT GTC TAG ACATTGATGA 1585
Tyr Arg Lys Ser Tyr Met Ala Ala Thr Val Val
1025 1030 1035

AATATAGATG GTGCACAAAT CTTTGTGATT GTGGATTTGT GAAAATAGTA TTGCAATATG 1645

TTACTGAAGA AACTTTTCCT TATCCACTTA TAAGTGGAAA TAGGAAGAAT GTGTATATAT 1705

GTAAGGGGTG ACAATTATTT TGAAATAAAA TTAAGAAAAT AAC 1748

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 518 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Asp Met Ala Phe Val Glu Val Cys Leu Arg Met Leu Leu Val Phe
  1               5                  10                  15

Val Leu Ser Trp Thr Ile Phe His Val Asn Asn Arg Lys Lys Lys Lys
             20                  25                  30

Ala Thr Lys Leu Ala Asp Leu Ala Thr Glu Glu Arg Lys Glu Gly Gly
         35                  40                  45

Pro Asp Val Ile Ile Val Gly Ala Gly Val Gly Gly Ser Ala Leu Ala
     50                  55                  60

Tyr Ala Leu Ala Lys Asp Gly Arg Arg Val His Val Ile Glu Arg Asp
 65                  70                  75                  80

Met Arg Glu Pro Val Arg Met Met Gly Glu Phe Met Gln Pro Gly Gly
                 85                  90                  95

Arg Leu Met Leu Ser Lys Leu Gly Leu Gln Asp Cys Leu Glu Glu Ile
            100                 105                 110

Asp Ala Gln Lys Ser Thr Gly Ile Arg Leu Phe Lys Asp Gly Lys Glu
        115                 120                 125
```

Thr Val Ala Cys Phe Pro Val Asp Thr Asn Phe Pro Tyr Glu Pro Ser
130 135 140

Gly Arg Phe Phe His Asn Gly Arg Phe Val Gln Arg Leu Arg Gln Lys
145 150 155 160

Ala Ser Ser Leu Pro Asn Val Arg Leu Glu Glu Gly Thr Val Arg Ser
165 170 175

Leu Ile Glu Glu Lys Gly Val Val Lys Gly Val Thr Tyr Lys Asn Ser
180 185 190

Ser Gly Glu Glu Thr Thr Ser Phe Ala Pro Leu Thr Val Val Cys Asp
195 200 205

Gly Cys His Ser Asn Leu Arg Arg Ser Leu Asn Asp Asn Asn Ala Glu
210 215 220

Val Thr Ala Tyr Glu Ile Gly Tyr Ile Ser Arg Asn Cys Arg Leu Glu
225 230 235 240

Gln Pro Asp Lys Leu His Leu Ile Met Ala Lys Pro Ser Phe Ala Met
245 250 255

Leu Tyr Gln Val Ser Ser Thr Asp Val Arg Cys Asn Phe Glu Leu Leu
260 265 270

Ser Lys Asn Leu Pro Ser Val Ser Asn Gly Glu Met Thr Ser Phe Val
275 280 285

Arg Asn Ser Ile Ala Pro Gln Val Pro Leu Lys Leu Arg Lys Thr Phe
290 295 300

Leu Lys Gly Leu Asp Glu Gly Ser His Ile Lys Ile Thr Gln Ala Lys
305 310 315 320

Arg Ile Pro Ala Thr Leu Ser Arg Lys Lys Gly Val Ile Val Leu Gly
325 330 335

Asp Ala Phe Asn Met Arg His Pro Val Ile Ala Ser Gly Met Met Val
340 345 350

Leu Leu Ser Asp Ile Leu Ile Leu Ser Arg Leu Leu Lys Pro Leu Gly
355 360 365

Asn Leu Gly Asp Glu Asn Lys Val Ser Glu Val Met Lys Ser Phe Tyr
370 375 380

Ala Leu Arg Lys Pro Met Ser Ala Thr Val Asn Thr Leu Gly Asn Ser
385 390 395 400

Phe Trp Gln Val Leu Ile Ala Ser Thr Asp Glu Ala Lys Glu Ala Met
405 410 415

Arg Gln Gly Cys Phe Asp Tyr Leu Ser Ser Gly Gly Phe Arg Thr Ser
420 425 430

Gly Leu Met Ala Leu Ile Gly Gly Met Asn Pro Arg Pro Leu Ser Leu
435 440 445

Phe Tyr His Leu Phe Val Ile Ser Leu Ser Ser Ile Gly Gln Leu Leu
450 455 460

Ser Pro Phe Pro Thr Pro Leu Arg Val Trp His Ser Leu Arg Leu Leu
465 470 475 480

Asp Leu Ser Leu Lys Met Leu Val Pro His Leu Lys Ala Glu Gly Ile
485 490 495

Gly Gln Met Leu Ser Pro Thr Asn Ala Ala Ala Tyr Arg Lys Ser Tyr
500 505 510

Met Ala Ala Thr Val Val
515

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1893 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Bassica napus

(B) STRAIN: Westar

(D) DEVELOPMENTAL STAGE: 14 day greening-etiolated

(F) TISSUE TYPE: hypocotyls

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Tsang

(B) CLONE: pDR411

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION:1..28

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION:29..1466

(ix) FEATURE:

(A) NAME/KEY: intron

(B) LOCATION:1467..1623

(ix) FEATURE:

(A) NAME/KEY: exon

(B) LOCATION:1624..1697

(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION:1698..1893

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CCACGCGTCC GCGGACGCGT GGGCAGATAT GGATCTAGCT TTTCCGCACG TTTGTTTGTG 60

GACGCTACTC GCCTTTGTGC TGACTTGGAC AGTGTTCTAC GTCAACAACA GGAGGAAGAA 120

GGTGGCGAAG TTACCCGATG CGGCGACAGA GGTGAGAAGA GACGGTGATG CTGACGTCAT 180

CATCGTCGGA GCTGGTGTTG GAGGTTCAGC TCTCGCCTAC GCTCTTGCAA AGGATGGGCG 240

TCGAGTACAT GTGATAGAGA GGGACATGAG GGAACCAGTG AGAATGATGG GTGAATTTAT 300

GCAACCCGGT GGACGACTAC TGCTTTCTAA GCTTGGTCTT GAAGATTGTT TGGAGGGAAT 360

AGATGAACAG ATAGCCACAG GCTTAGCAGT TTATAAGGAC GGACAAAAAG CACTCGTGTC 420

TTTTCCAGAG GACAACGACT TTCCTTATGA ACCTACTGGT CGAGCTTTTT ATAATGGCCG 480

TTTTGTCCAG AGACTGCGCC AAAAGGCTTC TTCGCTCCCC ACTGTACAAC TTGAAGAAGG 540

GACTGTAAAA TCTTTGATAG AAGAAAAAGG AGTGATCAAA GGAGTGACAT ACAAGAATAG 600

TGCAGGCGAA GAAACGACTG CATTTGCACC TCTCACAGTG GTATGCGACG GTTGCTATTC 660

AAACCTTCGT CGGTCTGTTA ACGACAACAA TGCGGAGGTT ATATCGTACC AAGTTGGTTA 720

CGTCTCAAAG AATTGTCAGC TTGAAGATCC TGAAAAGTTA AAATTGATAA TGTCTAAACC 780

TTCCTTCACC ATGTTGTATC AAATAAGCAG CACCGATGTT CGTTGTGTTA TGGAGATTTT 840

CCCCGGCAAT ATTCCTTCTA TTTCAAATGG CGAAATGGCT GTTTATTTGA AAAATACTAT 900

GGCTCCTCAG GTACCTCCAG AACTCCGCAA AATATTTTTG AAAGGAATTG ATGAGGGAGC 960

ACAAATTAAA GCGATGCCAA CAAAGAGAAT GGAAGCTACT TTGAGCGAAA AGCAAGGAGT 1020

GATTGTGTTG GGAGATGCAT TCAACATGCG CCACCCAGCG ATTGCCTCTG GAATGATGGT 1080

TGTATTATCT GACATTCTCA TTCTACGCCG CCTTCTCCAG CCATTGCGAA ACCTCAGTGA 1140

TGCAAATAAA GTATCAGAAG TTATTAAGTC ATTTTATGTC ATCCGAAAGC CAATGTCAGC 1200

GACGGTGAAC ACGCTAGGAA ATGCATTTTC TCAAGTGCTA ATTGCATCTA CGGACGAAGC 1260

AAAAGAAGCG ATGCGACAAG GCTGTTTTGA TTACCTCTCT AGTGGCGGCT TTCGCACGTC 1320

AGGAATGATG GCTCTGCTCG GTGGCATGAA CCCTCGACCA CTCTCTCTCA TCTTTCATCT 1380

ATGTGGTATT ACTCTATCCT CCATTGGTCA ACTGCTCTCG CCATTTCCAT CTCCTCTTGG 1440

CATTTGGCAT AGCCTCAGAC TTTTTGGTGT AAGTCATTAT CTCCCTCCCT ATGTTATTTA 1500

CATATTTTTC TTTGTGTTAT ATATTTTGTA AATAATTTAC AATTGAATTT TGACATTTTC 1560

TTGTTGTTTA TGTGTATGCC TAATTGTCTA TGAAAATGTT GGTTCCTCAT CTTAAGGCTG 1620

AAGGGGTTAG CCAAATGCTG TCTCCAGCAT ACGCAGCCGC GTATCGCAAA AGCTATATGA 1680

CCGCAACCGC TCTCTAAGCA TCGATGATAA GAACCGCGAA TGATACTATG ACATATTTGG 1740

AGCGCTAGTA TTTTGTGGTT TTGCATCCGT TAAAAATTTA AAATGTGTTG CTGTGTGTTT 1800

ACTATTATTA GTGTATTACC TGGAAAATAC CCGTGGGTAT ATTCTAAATG TATAAAATAT 1860

TGTGATAAAT AAAACGACTC TCCGTTTGGT TGG 1893

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 572 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Mus Musculus

(B) STRAIN: B6CBA

(D) DEVELOPMENTAL STAGE: 6-8 weeks

(F) TISSUE TYPE: liver

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: Lambda ZAP vector Stratagene catalog #935302

(B) CLONE: pMMSE-17

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Kosuga, K.
Hata, S.
Osumi, T.
Sakakibara, J.
Ono, T.

(B) TITLE: Nucleotide sequence of a cDNA for mouse squalene epoxidase

(C) JOURNAL: Biochim. Biophys. Acta

(D) VOLUME: 1260

(E) ISSUE: 3

(F) PAGES: 345-348

(G) DATE: 1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Trp Thr Phe Leu Gly Ile Ala Thr Phe Thr Tyr Phe Tyr Lys Lys
1               5                   10                  15

Cys Gly Asp Val Thr Leu Ala Asn Lys Glu Leu Leu Leu Cys Val Leu
            20                  25                  30

Val Phe Leu Ser Leu Gly Leu Val Leu Ser Tyr Arg Cys Arg His Arg
        35                  40                  45

His Gly Gly Leu Leu Gly Arg His Gln Ser Gly Ala Gln Phe Ala Ala
    50                  55                  60

Phe Ser Asp Ile Leu Ser Ala Leu Pro Leu Ile Gly Phe Phe Trp Ala
65                  70                  75                  80

Lys Ser Pro Glu Ser Glu Lys Lys Glu Gln Leu Glu Ser Lys Lys Cys
```

85 90 95

Arg Lys Glu Ile Gly Leu Ser Glu Thr Thr Leu Thr Gly Ala Ala Thr
100 105 110

Ser Val Ser Thr Ser Phe Val Thr Asp Pro Glu Val Ile Ile Val Gly
115 120 125

Ser Gly Val Leu Gly Ser Ala Leu Ala Ala Val Leu Ser Arg Asp Gly
130 135 140

Arg Lys Val Thr Val Ile Glu Arg Asp Leu Lys Glu Pro Asp Arg Ile
145 150 155 160

Val Gly Glu Leu Leu Gln Pro Gly Gly Tyr Arg Val Leu Gln Glu Leu
165 170 175

Gly Leu Gly Asp Thr Val Glu Gly Leu Asn Ala His His Ile His Gly
180 185 190

Tyr Ile Val His Asp Tyr Glu Ser Arg Ser Glu Val Gln Ile Pro Tyr
195 200 205

Pro Leu Ser Glu Thr Asn Gln Val Gln Ser Gly Ile Ala Phe His His
210 215 220

Gly Arg Phe Ile Met Ser Leu Arg Lys Ala Ala Met Ala Glu Pro Asn
225 230 235 240

Val Lys Phe Ile Glu Gly Val Val Leu Gln Leu Leu Glu Glu Asp Asp
245 250 255

Ala Val Ile Gly Val Gln Tyr Lys Asp Lys Glu Thr Gly Asp Thr Lys
260 265 270

Glu Leu His Ala Pro Leu Thr Val Val Ala Asp Gly Leu Phe Ser Lys
275 280 285

Phe Arg Lys Ser Leu Ile Ser Ser Lys Val Ser Val Ser Ser His Phe
290 295 300

Val Gly Phe Leu Met Lys Asp Ala Pro Gln Phe Lys Pro Asn Phe Ala
305 310 315 320

Glu Leu Val Leu Val Asn Pro Ser Pro Val Leu Ile Tyr Gln Ile Ser
325 330 335

Ser Ser Glu Thr Arg Val Leu Val Asp Ile Arg Gly Glu Leu Pro Arg
340 345 350

Asn Leu Arg Glu Tyr Met Ala Glu Gln Ile Tyr Pro Gln Leu Pro Glu
355 360 365

His Leu Lys Glu Ser Phe Leu Glu Ala Ser Gln Asn Gly Arg Leu Arg
370 375 380

Thr Met Pro Ala Ser Phe Leu Pro Pro Ser Ser Val Asn Lys Arg Gly
385 390 395 400

Val Leu Ile Leu Gly Asp Ala Tyr Asn Leu Arg His Pro Leu Thr Gly
405 410 415

Gly Gly Met Thr Val Ala Leu Lys Asp Ile Lys Leu Trp Arg Gln Leu
420 425 430

Leu Lys Asp Ile Pro Asp Leu Tyr Asp Asp Ala Ala Ile Phe Gln Ala
435 440 445

Lys Lys Ser Phe Phe Trp Ser Arg Lys Arg Thr His Ser Phe Val Val
450 455 460

Asn Val Leu Ala Gln Ala Leu Tyr Glu Leu Phe Ser Ala Thr Asp Asp
465 470 475 480

Ser Leu His Gln Leu Arg Lys Ala Cys Phe Leu Tyr Phe Lys Leu Gly
485 490 495

Gly Glu Cys Val Thr Gly Pro Val Gly Leu Leu Ser Ile Leu Ser Pro
500 505 510

His Pro Leu Val Leu Ile Arg His Phe Phe Ser Val Ala Ile Tyr Ala
515 520 525

Thr Tyr Phe Cys Phe Lys Ser Glu Pro Trp Ala Thr Lys Pro Arg Ala
530 535 540

Leu Phe Ser Ser Gly Ala Val Leu Tyr Lys Ala Cys Ser Ile Leu Phe
545 550 555 560

Pro Leu Ile Tyr Ser Glu Met Lys Tyr Leu Val His
565 570

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus
(F) TISSUE TYPE: kidney
(H) CELL LINE: NRK

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: pcD2 library of H. Okayama
(B) CLONE: Tb-1

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Sakakibara, J.
Watanabe, R.
Kanai, R.

Ono, T.

(B) TITLE: Molecular cloning and expression of rat sqalene epoxidase

(C) JOURNAL: J. Biol. Chem.

(D) VOLUME: 270

(E) ISSUE: 1

(F) PAGES: 17-20

(G) DATE: 1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Trp Thr Phe Leu Gly Ile Ala Thr Phe Thr Tyr Phe Tyr Lys Lys
1               5                   10                  15

Cys Gly Asp Val Thr Leu Ala Asn Lys Glu Leu Leu Leu Cys Val Leu
            20                  25                  30

Val Phe Leu Ser Leu Gly Leu Val Leu Ser Tyr Arg Cys Arg His Arg
        35                  40                  45

Asn Gly Gly Leu Leu Gly Arg His Gln Ser Gly Ser Gln Phe Ala Ala
    50                  55                  60

Phe Ser Asp Ile Leu Ser Ala Leu Pro Leu Ile Gly Phe Phe Trp Ala
65                  70                  75                  80

Lys Ser Pro Pro Glu Ser Glu Lys Lys Glu Gln Leu Glu Ser Lys Arg
                85                  90                  95

Arg Arg Lys Glu Val Asn Leu Ser Glu Thr Thr Leu Thr Gly Ala Ala
            100                 105                 110

Thr Ser Val Ser Thr Ser Ser Val Thr Asp Pro Glu Val Ile Ile Ile
        115                 120                 125

Gly Ser Gly Val Leu Gly Ser Ala Leu Ala Thr Val Leu Ser Arg Asp
    130                 135                 140

Gly Arg Thr Val Thr Val Ile Glu Arg Asp Leu Lys Glu Pro Asp Arg
145                 150                 155                 160
```

Ile Leu Gly Glu Cys Leu Gln Pro Gly Gly Tyr Arg Val Leu Arg Glu
165 170 175

Leu Gly Leu Gly Asp Thr Val Glu Ser Leu Asn Ala His His Ile His
180 185 190

Gly Tyr Val Ile His Asp Cys Glu Ser Arg Ser Glu Val Gln Ile Pro
195 200 205

Tyr Pro Val Ser Glu Asn Asn Gln Val Gln Ser Gly Val Ala Phe His
210 215 220

His Gly Lys Phe Ile Met Ser Leu Arg Lys Ala Ala Met Ala Glu Pro
225 230 235 240

Asn Val Lys Phe Ile Glu Gly Val Val Leu Arg Leu Leu Glu Glu Asp
245 250 255

Asp Ala Val Ile Gly Val Gln Tyr Lys Asp Lys Glu Thr Gly Asp Thr
260 265 270

Lys Glu Leu His Ala Pro Leu Thr Val Val Ala Asp Gly Leu Phe Ser
275 280 285

Lys Phe Arg Lys Asn Leu Ile Ser Asn Lys Val Ser Val Ser Ser His
290 295 300

Phe Val Gly Phe Ile Met Lys Asp Ala Pro Gln Phe Lys Ala Asn Phe
305 310 315 320

Ala Glu Leu Val Leu Val Asp Pro Ser Pro Val Leu Ile Tyr Gln Ile
325 330 335

Ser Pro Ser Glu Thr Arg Val Leu Val Asp Ile Arg Gly Glu Leu Pro
340 345 350

Arg Asn Leu Arg Glu Tyr Met Thr Glu Gln Ile Tyr Pro Gln Ile Pro
355 360 365

Asp His Leu Lys Glu Ser Phe Leu Glu Ala Cys Gln Asn Ala Arg Leu
370 375 380

Arg Thr Met Pro Ala Ser Phe Leu Pro Pro Ser Ser Val Asn Lys Arg
385 390 395 400

Gly Val Leu Leu Leu Gly Asp Ala Tyr Asn Leu Arg His Pro Leu Thr
405 410 415

Gly Gly Gly Met Thr Val Ala Leu Lys Asp Ile Lys Ile Trp Arg Gln
420 425 430

Leu Leu Lys Asp Ile Pro Asp Leu Tyr Asp Asp Ala Ala Ile Phe Gln
435 440 445

Ala Lys Lys Ser Phe Phe Trp Ser Arg Lys Arg Ser His Ser Phe Val
450 455 460

Val Asn Val Leu Ala Gln Ala Leu Tyr Glu Leu Phe Ser Ala Thr Asp
465 470 475 480

Asp Ser Leu Arg Gln Leu Arg Lys Ala Cys Phe Leu Tyr Phe Lys Leu
485 490 495

Gly Gly Glu Cys Leu Thr Gly Pro Val Gly Leu Leu Ser Ile Leu Ser
500 505 510

Pro Asp Pro Leu Leu Leu Ile Arg His Phe Phe Ser Val Ala Val Tyr
515 520 525

Ala Thr Tyr Phe Cys Phe Lys Ser Glu Pro Trp Ala Thr Lys Pro Arg
530 535 540

Ala Leu Phe Ser Ser Gly Ala Ile Leu Tyr Lys Ala Cys Ser Ile Ile
545 550 555 560

Phe Pro Leu Ile Tyr Ser Glu Met Lys Tyr Leu Val His
565 570

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 496 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Saccharomyces cerevisiae

(B) STRAIN: A2-M8

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Jandrositz, A.
Hoegenauer, G.
Turnowsky, F.

(B) TITLE: The gene encoding squalene epoxidase from Saccharomyces cerevisiae: cloning and characterization

(C) JOURNAL: Gene

(D) VOLUME: 107

(F) PAGES: 155-160

(G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Met Ser Ala Val Asn Val Ala Pro Glu Leu Ile Asn Ala Asp Asn Thr
1 5 10 15

Ile Thr Tyr Asp Ala Ile Val Ile Gly Ala Gly Val Ile Gly Pro Cys
20 25 30

Val Ala Thr Gly Leu Ala Arg Lys Gly Lys Lys Val Leu Ile Val Glu
35 40 45

Arg Asp Trp Ala Met Pro Asp Arg Ile Val Gly Glu Leu Met Gln Pro
50 55 60

Gly Gly Val Arg Ala Leu Arg Ser Leu Gly Met Ile Gln Ser Ile Asn
65 70 75 80

Asn Ile Glu Ala Tyr Pro Val Thr Gly Tyr Thr Val Phe Phe Asn Gly
85 90 95

Glu Gln Val Asp Ile Pro Tyr Pro Tyr Lys Ala Asp Ile Pro Lys Val
100 105 110

Glu Lys Leu Lys Asp Leu Val Lys Asp Gly Asn Asp Lys Val Leu Glu
115 120 125

Asp Ser Thr Ile His Ile Lys Asp Tyr Glu Asp Asp Glu Arg Glu Arg
130 135 140

Gly Val Ala Phe Val His Gly Arg Phe Leu Asn Asn Leu Arg Asn Ile
145 150 155 160

Thr Ala Gln Glu Pro Asn Val Thr Arg Val Gln Gly Asn Cys Ile Glu
165 170 175

Ile Leu Lys Asp Glu Lys Asn Glu Val Val Gly Ala Lys Val Asp Ile
180 185 190

Asp Gly Arg Gly Lys Val Glu Phe Lys Ala His Leu Thr Phe Ile Cys
195 200 205

Asp Gly Ile Phe Ser Arg Phe Arg Lys Glu Leu His Pro Asp His Val
210 215 220

Pro Thr Val Gly Ser Ser Phe Val Gly Met Ser Leu Phe Asn Ala Lys
225 230 235 240

Asn Pro Ala Pro Met His Gly His Val Ile Phe Gly Ser Asp His Met
245 250 255

Pro Ile Leu Val Tyr Gln Ile Ser Pro Glu Glu Thr Arg Ile Leu Cys
260 265 270

Ala Tyr Asn Ser Pro Lys Val Pro Ala Asp Ile Lys Ser Trp Met Ile
275 280 285

Lys Asp Val Gln Pro Phe Ile Pro Lys Ser Leu Arg Pro Ser Phe Asp
290 295 300

Glu Ala Val Ser Gln Gly Lys Phe Arg Ala Met Pro Asn Ser Tyr Leu
305 310 315 320

Pro Ala Arg Gln Asn Asp Val Thr Gly Met Cys Val Ile Gly Asp Ala
325 330 335

Leu Asn Met Arg His Pro Leu Thr Gly Gly Gly Met Thr Val Gly Leu
340 345 350

His Asp Val Val Leu Leu Ile Lys Lys Ile Gly Asp Leu Asp Phe Ser
355 360 365

Asp Arg Glu Lys Val Leu Asp Glu Leu Leu Asp Tyr His Phe Glu Arg
370 375 380

Lys Ser Tyr Asp Ser Val Ile Asn Val Leu Ser Val Ala Leu Tyr Ser
385 390 395 400

Leu Phe Ala Ala Asp Ser Asp Asn Leu Lys Ala Leu Gln Lys Gly Cys
405 410 415

Phe Lys Tyr Phe Gln Arg Gly Gly Asp Cys Val Asn Lys Pro Val Glu
420 425 430

Phe Leu Ser Gly Val Leu Pro Lys Pro Leu Gln Leu Thr Arg Val Phe
435 440 445

Phe Ala Val Ala Phe Tyr Thr Ile Tyr Leu Asn Met Glu Glu Arg Gly
450 455 460

Phe Leu Gly Leu Pro Met Ala Leu Leu Glu Gly Ile Met Ile Leu Ile

465 470 475 480

Thr Ala Ile Arg Val Phe Thr Pro Phe Leu Phe Gly Glu Leu Ile Gly
485 490 495

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 536 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: Columbia
(D) DEVELOPMENTAL STAGE: 4 different stages and tissues

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Lambda-PRL2
(B) CLONE: 250F2T7

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Newman, T.
deBruijn, F. J.
Green, P.
Keegstra, K.
Kende, H.
McIntosh, L.
Ohlrogge, J.
Raikhel, N.
Somerville, S.
Thomashow, M.
(B) TITLE: Genes galore: a summary of methods for

accessing results from large-scale partial sequencing of anonymous Arabidopsis cDNA clones

(C) JOURNAL: Plant Physiol.

(D) VOLUME: 106

(F) PAGES: 1241-1255

(G) DATE: 1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
GAGAACATAT AAAAGCCATG CCAACAAAGA AGATGACAGC TACTTTGAGC GAGAAGAAAG      60

GAGTGATTTT ATTGGGAGAT GCATTCAACA TGCGTCATCC AGCAATCGCA TCTGGAATGA     120

TGGTTTTATT ATCTGACATT CTCATTCTAC GCCGTCTTCT CCAGCCATTA AGCAACCTTG     180

GCAATGCGCA AAAAATCTCA CAAGTTATCA AGTCCTTTTA TGATATCCGC AAGCCAATGT     240

CAGCGACAGT TAACACGTTA GGAAATGCAT TCTCTCAAGT GCTAGTTGCA TCGACGGACG     300

AAGCAAAAGA GGCAATGAGA CAAGGTTGCT ATGATTACCT CTCTAGTGGT GGGTTTCGCA     360

CGTCAGGGAT GATGGCTTTG CTAGGCGGAT GAACCCTCGT CCGATCTCTC NCATCNANCA     420

NCNAGGGGAA CACNCANCCC CATNGGCATC AACNCCNCAT TCCCNNCCCT TCGATTGGAA     480

CCTCGACTTT TGGTGGNNNA AAGGTGGCCC CCCANGGGAA GGTTCCATNT NTCCNC         536
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 540 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Ricinus Communis

(B) STRAIN: Baker 296

(D) DEVELOPMENTAL STAGE: immature castor fruits

(F) TISSUE TYPE: endosperm and embryo

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: lambdaZAPST

(B) CLONE: pcrs547

(x) PUBLICATION INFORMATION:

(A) AUTHORS: van de Loo, F. J.
Turner, S.
Somerville, C.

(B) TITLE: Expressed sequence tags from developing castor seeds

(C) JOURNAL: Plant Physiol.

(D) VOLUME: 108

(F) PAGES: 1141-1150

(G) DATE: 1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTTGAGCTCA GAGTCACAGA TATAGACATC CTAGGGAAAA CATTCTCCTA TAAACTAAAG 60

CGTATTACAA TTCACACTTC TTTTCCCCTC AACTTTGATT TGAACAAAGG GATGAGATTA 120

AAACCAAAAT GAGAAACGCC CCGTTCCTTC TTGTCACGAA TTTTTCACTC ACATTCTTGT 180

CAAACTAATT GCATTCAACA GGAGGAGCTC TATAATATGC TGGGACGGTT GCGGGGAAGA 240

ACATCTGTCT AACTCCTTCT GCCTTGATAA TGGGGAAGAT GATTCCTGAT GCACCCGATA 300

TCAACCTAGC TCCAACCCAG ACGCGCTTAG GTGAAGGGAA TGGCAGTAAC AAAGGGGGGG 360

CCCGGTACCC AATTTGCCCT ATAGTGAGCC GTATTCAATN ACTGGCCGTT GTTTCAACGT 420

GTGCCTTGGG AAACCCTGGG GTNCCACTTA TTGCTTCAGA CATCCCCTTT GCANTTGGTA 480

TTNGAGGGGC CGACCGTTGC CTCCAANAGT NCNCGTTNAA TTGGGTTGAA ANTTNCGGGA 540

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 503 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Met Asp Leu Ala Phe Pro His Val Cys Leu Trp Thr Leu Leu Ala Phe
1               5                   10                  15

Val Leu Thr Trp Thr Val Phe Tyr Val Asn Asn Arg Arg Lys Lys Val
            20                  25                  30

Ala Lys Leu Pro Asp Ala Ala Thr Glu Val Arg Arg Asp Gly Asp Ala
        35                  40                  45

Asp Val Ile Ile Val Gly Ala Gly Val Gly Gly Ser Ala Leu Ala Tyr
    50                  55                  60

Ala Leu Ala Lys Asp Gly Arg Arg Val His Val Ile Glu Arg Asp Met
65                  70                  75                  80

Arg Glu Pro Val Arg Met Met Gly Glu Phe Met Gln Pro Gly Gly Arg
                85                  90                  95

Leu Leu Leu Ser Lys Leu Gly Leu Glu Asp Cys Leu Glu Gly Ile Asp
            100                 105                 110
```

Glu Gln Ile Ala Thr Gly Leu Ala Val Tyr Lys Asp Gly Gln Lys Ala
115 120 125

Leu Val Ser Phe Pro Glu Asp Asn Asp Phe Pro Tyr Glu Pro Thr Gly
130 135 140

Arg Ala Phe Tyr Asn Gly Arg Phe Val Gln Arg Leu Arg Gln Lys Ala
145 150 155 160

Ser Ser Leu Pro Thr Val Gln Leu Glu Glu Gly Thr Val Lys Ser Leu
165 170 175

Ile Glu Glu Lys Gly Val Ile Lys Gly Val Thr Tyr Lys Asn Ser Ala
180 185 190

Gly Glu Glu Thr Thr Ala Phe Ala Pro Leu Thr Val Val Cys Asp Gly
195 200 205

Cys Tyr Ser Asn Leu Arg Arg Ser Val Asn Asp Asn Asn Ala Glu Val
210 215 220

Ile Ser Tyr Gln Val Gly Tyr Val Ser Lys Asn Cys Gln Leu Glu Asp
225 230 235 240

Pro Glu Lys Leu Lys Leu Ile Met Ser Lys Pro Ser Phe Thr Met Leu
245 250 255

Tyr Gln Ile Ser Ser Thr Asp Val Arg Cys Val Met Glu Ile Phe Pro
260 265 270

Gly Asn Ile Pro Ser Ile Ser Asn Gly Glu Met Ala Val Tyr Leu Lys
275 280 285

Asn Thr Met Ala Pro Gln Val Pro Pro Glu Leu Arg Lys Ile Phe Leu
290 295 300

Lys Gly Ile Asp Glu Gly Ala Gln Ile Lys Ala Met Pro Thr Lys Arg
305 310 315 320

Met Glu Ala Thr Leu Ser Glu Lys Gln Gly Val Ile Val Leu Gly Asp

325 330 335

Ala Phe Asn Met Arg His Pro Ala Ile Ala Ser Gly Met Met Val Val
340 345 350

Leu Ser Asp Ile Leu Ile Leu Arg Arg Leu Leu Gln Pro Leu Arg Asn
355 360 365

Leu Ser Asp Ala Asn Lys Val Ser Glu Val Ile Lys Ser Phe Tyr Val
370 375 380

Ile Arg Lys Pro Met Ser Ala Thr Val Asn Thr Leu Gly Asn Ala Phe
385 390 395 400

Ser Gln Val Leu Ile Ala Ser Thr Asp Glu Ala Lys Glu Ala Met Arg
405 410 415

Gln Gly Cys Phe Asp Tyr Leu Ser Ser Gly Gly Phe Arg Thr Ser Gly
420 425 430

Met Met Ala Leu Leu Gly Gly Met Asn Pro Arg Pro Leu Ser Leu Ile
435 440 445

Phe His Leu Cys Gly Ile Thr Leu Ser Ser Ile Gly Gln Leu Leu Ser
450 455 460

Pro Phe Pro Ser Pro Leu Gly Ile Trp His Ser Leu Arg Leu Phe Gly
465 470 475 480

Val Ser Gln Met Leu Ser Pro Ala Tyr Ala Ala Ala Tyr Arg Lys Ser
485 490 495

Tyr Met Thr Ala Thr Ala Leu
500

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Bondioli, P., Mariani, C., Lanzani, A., Fedeli, E. and Muller, A. (1993) Squalene recovery from olive oil deodorizer distillates. *J. Am. Oil Chem. Soc.* 70, 763-766.

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concomitant reduction of gene expression in vivo. EMBO J. 11, 1525-1530.

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The teachings of the above references are specifically incorporated herein by reference.

CLAIMS:

1. An isolated and cloned DNA suitable for introduction into a genome of a plant to suppress expression of squalene epoxidase by said plant below natural levels, characterised in that said DNA has a sequence corresponding at least in part to a squalene epoxidase gene of a plant.

2. DNA according to claim 1, characterised by a sequence corresponding to all or part of a specific sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10; or having at least 60% homology thereto.

3. DNA according to claim 2, characterised in that said part of said sequence comprises at least 20 consecutive nucleotides of said specific sequence.

4. DNA according to claim 2, characterised in that said part of said sequence comprises at least 100 consecutive nucleotides of said specific sequence.

5. A process of producing genetically-modified plants having increased levels of squalene in tissues of the plants compared to corresponding wild-type plants, wherein the plant genome is modified to suppress expression of squalene expoxidase by said plant, characterised in that said genome is modified by introducing at least one exogenous DNA sequence that corresponds, at least in part, to one or more endogenous squalene epoxidase genes of said plant.

6. A process according to claim 5, characterised in that said DNA sequence introduced into said plant genome has at least 60% homology to said one or more of said

endogenous squalene epoxidase genes.

7. A process according to claim 5, characterised in that said exogenous DNA has a sequence corresponding to all or part of a specific sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10; or has at least 60% homology thereto.

8. A process according to claim 7, characterised in that said part of said sequence comprises at least 20 consecutive nucleotides of said specific sequence.

9. A process according to claim 7, characterised in that said part of said sequence comprises at least 100 consecutive nucleotides of said specific sequence.

10. A process as claimed in claim 5, characterised in that said at least one DNA sequence introduced into said genome is arranged in a sense orientation relative to a transcriptional promoter such that it is capable of decreasing said expression by co-suppression or homology-dependent gene silencing.

11. A process as claimed in claim 5, characterised in that said at least one DNA sequence introduced into said genome forms part of a gene encoding a ribozyme that is capable of catalysing endonucleolytic cleavage of said one or more of the endogenous squalene epoxidase genes of said plant.

12. A process as claimed in claim 5, characterised in that said exogenous DNA is obtained by identifying at least one squalene epoxidase gene of said plant, and sequencing and cloning the gene or at least a part thereof.

13. A process according to claim 5, characterised in that said exogenous DNA sequence is introduced into said plant by a procedure selected from *Abrobacterium*-mediated and particle gun transformation techniques.

14. A process of producing genetically-modified plants having increased levels of squalene in tissues of the plants compared to corresponding wild-type plants, wherein the plant genome is modified to suppress expression of squalene expoxidase by said plant by introducing a nucleotide sequence that reduces or prevents expression of squalene epoxidase into a genome of said plant, characterised in that said DNA includes a transcriptional promoter and a sequence arranged such that when transcribed from the promoter, resulting RNA is complementary or antisense to all or part of at least one squalene epoxidase messenger RNA transcribed from a squalene epoxidase gene of said plant.

15. A process according to claim 14, characterised in that said nucleotide sequence comprises all or part of a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10; or is a sequence having at least 60% homology thereto.

16. Plasmid pDR411 (ATCC 97845).

17. Plasmid pDR111 (ATCC 97846).

18. Plasmid p129F12T7 (ATCC 97847).

19. A vector for introducing a nucleotide sequence into a plant genome, characterised in that said vector comprises a construct containing a nucleotide sequence that is antisense to a plant squalene epoxidase gene or a

part thereof, positioned between a transcriptional promoter segment and a transcriptional termination segment.

20. A vector according to claim 19, characterised in that said nucleotide sequence comprises all or part of a specific sequence selected from SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:9 and SEQ ID NO:10; or has at least 60% homology thereto.

21. Vector pSE129A (ATCC 97910).

22. Vector pSE411A (ATCC 97908).

23. Vector pSE111A (ATCC 97909).

24. A genetically-modified plant capable of accumulating squalene at levels higher than the corresponding wild-type plant, characterised in that said genetically-modified plant has been produced by a process according to claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, claim 12, claim 13, claim 14 or claim 15.

25. A seed of a genetically-modified oilseed plant containing squalene at levels higher than seeds of equivalent wild-type plants, characterised in that said genetically-modified plant has been produced by a process according to claim 5, claim 6, claim 7, claim 8, claim 9, claim 10, claim 11, claim 12, claim 13, claim 14 or claim 15.

26. A process of producing squalene, characterised by growing a genetically-modified plant as defined in claim 24, harvesting said plant or seeds of said plant, and extracting squalene from said harvested plant or seeds.

# FIG. 1A



10 20

| | | |
|---|---|---|
| B. NAPUS 111 | M - - - - - - - - - - - - - - - - - - - | 2 |
| B. NAPUS 411 | M - - - - - - - - - - - - - - - - - - - | 2 |
| ARABIDOPSIS | M - - - - - - - - - - - - - - - - - - - | 2 |
| MOUSE | M W T F L G I A T F T Y F Y K K C G D V | 20 |
| RAT | M W T F L G I A T F T Y F Y K K C G D V | 20 |
| YEAST | M - - - - - - - - - - - - - - - - - - - | 2 |

30 40

| | | |
|---|---|---|
| B. NAPUS 111 | - - - - - - - - - - - - - - - - - - - - | 2 |
| B. NAPUS 411 | - - - - - - - - - - - - - - - - - - - - | 2 |
| ARABIDOPSIS | - - - - - - - - - - - - - - - - - - - - | 2 |
| MOUSE | T L A N K E L L L C V L V F L S L G L V | 40 |
| RAT | T L A N K E L L L C V L V F L S L G L V | 40 |
| YEAST | - - - - - - - - - - - - - - - - - - - - | 2 |

50 60

| | | |
|---|---|---|
| B. NAPUS 111 | - - - - - - - - - - - - - - - - - - - - | 2 |
| B. NAPUS 411 | - - - - - - - - - - - - - - - - - - - - | 2 |
| ARABIDOPSIS | - - - - - - - - - - - - - - - - - - - - | 2 |
| MOUSE | L S Y R C R H R H G G L L G R H Q S G A | 60 |
| RAT | L S Y R C R H R N G G L L G R H Q S G S | 60 |
| YEAST | - - - - - - - - - - - - - - - - - - - - | 2 |

70 80

| | | |
|---|---|---|
| B. NAPUS 111 | D M A F V E V C L R M L L V F V L S W T | 21 |
| B. NAPUS 411 | D L A F P H V C L W T L L A F V L T W T | 21 |
| ARABIDOPSIS | T Y A W - - - - L W T L L A F V L T W M | 17 |
| MOUSE | Q F A A F S D I L S A L P L I G F F W A | 80 |
| RAT | Q F A A F S D I L S A L P L I G F F W A | 80 |
| YEAST | - - - - - - - - - - - - - - - - - - - - | 2 |

90 100

| | | |
|---|---|---|
| B. NAPUS 111 | I F - - - - - - - - H V N N R K K K K A | 33 |
| B. NAPUS 411 | V F - - - - - - - - Y V N N R R K K - V | 32 |
| ARABIDOPSIS | V F - - - - - - - - H L I K M K K A A T | 29 |
| MOUSE | K S P - E S E K K E Q L E S K K C R K E | 99 |
| RAT | K S P P E S E K K E Q L E S K R R R K E | 100 |
| YEAST | - - - - - - - - - - - - - - - - - - - - | 2 |

110 120

| | | |
|---|---|---|
| B. NAPUS 111 | T K L A D L A T E E R K E G - - - - - - | 48 |
| B. NAPUS 411 | A K L P D A A T E V R R D G - - - - - - | 47 |
| ARABIDOPSIS | G D L - E A E A E A R R D G - - - - - - | 43 |
| MOUSE | I G L S E T T L T G A A T S V S T S F V | 119 |
| RAT | V N L S E T T L T G A A T S V S T S S V | 120 |
| YEAST | - - - - - S A V N V A P E L I N A D N T | 16 |

130 140

| | | |
|---|---|---|
| B. NAPUS 111 | - G P D V I I V G A G V G G S A L A Y A | 66 |
| B. NAPUS 411 | - D A D V I I V G A G V G G S A L A Y A | 65 |
| ARABIDOPSIS | - A T D V I I V G A G V A G A S L A Y A | 61 |
| MOUSE | T D P E V I I V G S G V L G S A L A A V | 139 |
| RAT | T D P E V I I I G S G V L G S A L A T V | 140 |
| YEAST | I T Y D A I V I G A G V I G P C V A T G | 36 |

# FIG. 1B



| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 150 | | | | | | | | | | 160 | |
| B. NAPUS 111 | L | A | K | D | G | R | R | V | H | V | I | E | R | D | M | R | E | P | V | R | 86 |
| B. NAPUS 411 | L | A | K | D | G | R | R | V | H | V | I | E | R | D | M | R | E | P | V | R | 85 |
| ARABIDOPSIS | L | A | K | D | G | R | R | V | H | V | I | E | R | D | L | K | E | P | Q | R | 81 |
| MOUSE | L | S | R | D | G | R | K | V | T | V | I | E | R | D | L | K | E | P | D | R | 159 |
| RAT | L | S | R | D | G | R | T | V | T | V | I | E | R | D | L | K | E | P | D | R | 160 |
| YEAST | L | A | R | K | G | K | K | V | L | I | V | E | R | D | W | A | M | P | D | R | 56 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 170 | | | | | | | | | | 180 | |
| B. NAPUS 111 | M | M | G | E | F | M | Q | P | G | G | R | L | M | L | S | K | L | G | L | Q | 106 |
| B. NAPUS 411 | M | M | G | E | F | M | Q | P | G | G | R | L | L | L | S | K | L | G | L | E | 105 |
| ARABIDOPSIS | F | M | G | E | L | M | Q | A | G | G | R | F | M | L | A | Q | L | G | L | E | 101 |
| MOUSE | I | V | G | E | L | L | Q | P | G | G | Y | R | V | L | Q | E | L | G | L | G | 179 |
| RAT | I | L | G | E | C | L | Q | P | G | G | Y | R | V | L | R | E | L | G | L | G | 180 |
| YEAST | I | V | G | E | L | M | Q | P | G | G | V | R | A | L | R | S | L | G | M | I | 76 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 190 | | | | | | | | | | 200 | |
| B. NAPUS 111 | D | C | L | E | E | I | D | A | Q | K | S | T | G | I | R | L | F | K | D | G | 126 |
| B. NAPUS 411 | D | C | L | E | G | I | D | E | Q | I | A | T | G | L | A | V | Y | K | D | G | 125 |
| ARABIDOPSIS | D | C | L | E | D | I | D | A | Q | E | A | K | S | L | A | I | Y | K | D | G | 121 |
| MOUSE | D | T | V | E | G | L | N | A | H | H | I | H | G | Y | I | V | H | D | Y | E | 199 |
| RAT | D | T | V | E | S | L | N | A | H | H | I | H | G | Y | V | I | H | D | C | E | 200 |
| YEAST | Q | S | I | N | N | I | E | A | Y | P | V | T | G | Y | T | V | F | F | N | G | 96 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 210 | | | | | | | | | | 220 | |
| B. NAPUS 111 | K | E | T | V | A | C | F | P | V | D | T | N | F | P | - | - | - | - | - | - | 141 |
| B. NAPUS 411 | Q | K | A | L | V | S | F | P | E | D | N | D | F | P | - | - | - | - | - | - | 140 |
| ARABIDOPSIS | K | H | A | T | L | P | F | P | D | D | K | S | F | P | - | - | - | - | - | - | 136 |
| MOUSE | S | R | S | E | V | Q | I | P | Y | P | L | S | - | - | - | - | - | - | - | - | 212 |
| RAT | S | R | S | E | V | Q | I | P | Y | P | V | S | - | - | - | - | - | - | - | - | 213 |
| YEAST | E | Q | V | D | I | P | Y | P | Y | K | A | D | I | P | K | V | E | K | L | K | 116 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 230 | | | | | | | | | | 240 | |
| B. NAPUS 111 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 141 |
| B. NAPUS 411 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 140 |
| ARABIDOPSIS | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 136 |
| MOUSE | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 212 |
| RAT | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 213 |
| YEAST | D | L | V | K | D | G | N | D | K | V | L | E | D | S | T | I | H | I | K | D | 136 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 250 | | | | | | | | | | 260 | |
| B. NAPUS 111 | - | - | - | - | Y | E | P | S | G | R | F | F | H | N | G | R | F | V | Q | R | 156 |
| B. NAPUS 411 | - | - | - | - | Y | E | P | T | G | R | A | F | Y | N | G | R | F | V | Q | R | 155 |
| ARABIDOPSIS | - | - | - | - | H | E | P | V | G | R | L | L | R | N | G | R | L | V | Q | R | 151 |
| MOUSE | - | E | T | N | Q | V | Q | S | G | I | A | F | H | H | G | R | F | I | M | S | 230 |
| RAT | - | E | N | N | Q | V | Q | S | G | V | A | F | H | H | G | K | F | I | M | S | 231 |
| YEAST | Y | E | D | D | E | R | E | R | G | V | A | F | V | H | G | R | F | L | N | N | 156 |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 270 | | | | | | | | | | 280 | |
| B. NAPUS 111 | L | R | Q | K | A | S | S | L | P | N | V | R | L | E | E | G | T | V | R | S | 176 |
| B. NAPUS 411 | L | R | Q | K | A | S | S | L | P | T | V | Q | L | E | E | G | T | V | K | S | 175 |
| ARABIDOPSIS | L | R | Q | K | A | A | S | L | S | N | V | Q | L | E | E | G | T | V | K | S | 171 |
| MOUSE | L | R | K | A | A | M | A | E | P | N | V | K | F | I | E | G | V | V | L | Q | 250 |
| RAT | L | R | K | A | A | M | A | E | P | N | V | K | F | I | E | G | V | V | L | R | 251 |
| YEAST | L | R | N | I | T | A | Q | E | P | N | V | T | R | V | Q | G | N | C | I | E | 176 |

# FIG. 1C

3/8

| | | | | | | | | | | 290 | | | | | | | | | | 300 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | L | I | E | E | K | G | V | V | K | G | V | T | Y | K | N | S | S | G | E | E | 196 |
| B. NAPUS 411 | L | I | E | E | K | G | V | I | K | G | V | T | Y | K | N | S | A | G | E | E | 195 |
| ARABIDOPSIS | L | I | E | E | E | G | V | V | K | G | V | T | Y | K | N | S | A | G | E | E | 191 |
| MOUSE | L | L | E | E | D | D | A | V | I | G | V | Q | Y | K | D | K | E | T | G | D | 270 |
| RAT | L | L | E | E | D | D | A | V | I | G | V | Q | Y | K | D | K | E | T | G | D | 271 |
| YEAST | I | L | K | D | E | K | N | E | V | V | G | A | K | V | D | I | D | G | R | G | 196 |

| | | | | | | | | | | 310 | | | | | | | | | | 320 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | T | T | S | F | - | A | P | L | T | V | V | C | D | G | C | H | S | N | L | R | 215 |
| B. NAPUS 411 | T | T | A | F | - | A | P | L | T | V | V | C | D | G | C | Y | S | N | L | R | 214 |
| ARABIDOPSIS | I | T | A | F | - | A | P | L | T | V | V | C | D | G | C | Y | S | N | L | R | 210 |
| MOUSE | T | K | E | L | H | A | P | L | T | V | V | A | D | G | L | F | S | K | F | R | 290 |
| RAT | T | K | E | L | H | A | P | L | T | V | V | A | D | G | L | F | S | K | F | R | 291 |
| YEAST | K | V | E | F | K | A | H | L | T | F | I | C | D | G | I | F | S | R | F | R | 216 |

| | | | | | | | | | | 330 | | | | | | | | | | 340 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | R | S | L | N | D | N | N | A | E | V | T | A | Y | E | - | I | G | Y | I | S | 234 |
| B. NAPUS 411 | R | S | V | N | D | N | N | A | E | V | I | S | Y | Q | - | V | G | Y | V | S | 233 |
| ARABIDOPSIS | R | S | L | V | D | N | T | E | E | V | L | S | Y | M | - | V | G | Y | V | T | 229 |
| MOUSE | K | S | L | I | S | S | K | V | S | - | V | S | S | H | F | V | G | F | L | M | 309 |
| RAT | K | N | L | I | S | N | K | V | S | - | V | S | S | H | F | V | G | F | I | M | 310 |
| YEAST | K | E | L | H | P | D | H | V | P | T | V | G | S | S | F | V | G | M | S | L | 236 |

| | | | | | | | | | | 350 | | | | | | | | | | 360 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | R | N | C | R | L | E | Q | P | D | K | L | H | L | I | M | - | A | K | P | S | 253 |
| B. NAPUS 411 | K | N | C | Q | L | E | D | P | E | K | L | K | L | I | M | - | S | K | P | S | 252 |
| ARABIDOPSIS | K | N | S | R | L | E | D | P | H | S | L | H | L | I | F | - | S | K | P | L | 248 |
| MOUSE | K | D | A | P | Q | F | K | P | N | F | A | E | L | V | L | V | - | N | P | S | 328 |
| RAT | K | D | A | P | Q | F | K | A | N | F | A | E | L | V | L | V | - | D | P | S | 329 |
| YEAST | F | N | A | K | N | P | A | P | M | H | G | H | V | I | F | G | S | D | H | M | 256 |

| | | | | | | | | | | 370 | | | | | | | | | | 380 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | F | A | M | L | Y | Q | V | S | S | T | D | V | R | C | N | F | E | L | L | S | 273 |
| B. NAPUS 411 | F | T | M | L | Y | Q | I | S | S | T | D | V | R | C | V | M | E | I | F | P | 272 |
| ARABIDOPSIS | V | C | V | I | Y | Q | I | T | S | D | E | V | R | C | V | A | E | V | P | A | 268 |
| MOUSE | P | V | L | I | Y | Q | I | S | S | S | E | T | R | V | L | V | D | I | R | G | 348 |
| RAT | P | V | L | I | Y | Q | I | S | P | S | E | T | R | V | L | V | D | I | R | G | 349 |
| YEAST | P | I | L | V | Y | Q | I | S | P | E | E | T | R | I | L | C | A | Y | N | S | 276 |

| | | | | | | | | | | 390 | | | | | | | | | | 400 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | K | N | L | P | S | V | S | N | G | E | M | T | S | F | V | R | N | S | I | A | 293 |
| B. NAPUS 411 | G | N | I | P | S | I | S | N | G | E | M | A | V | Y | L | K | N | T | M | A | 292 |
| ARABIDOPSIS | D | S | I | P | S | I | S | N | G | E | M | S | T | F | L | K | K | S | M | A | 288 |
| MOUSE | - | E | L | P | R | - | - | - | - | N | L | R | E | Y | M | A | E | Q | I | Y | 363 |
| RAT | - | E | L | P | R | - | - | - | - | N | L | R | E | Y | M | T | E | Q | I | Y | 364 |
| YEAST | P | K | V | P | A | - | - | - | - | D | I | K | S | W | M | I | K | D | V | Q | 292 |

| | | | | | | | | | | 410 | | | | | | | | | | 420 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | P | Q | V | P | L | - | - | K | L | R | K | T | F | L | K | G | L | D | E | G | 311 |
| B. NAPUS 411 | P | Q | V | P | P | - | - | E | L | R | K | I | F | L | K | G | I | D | E | G | 310 |
| ARABIDOPSIS | P | Q | I | P | E | T | G | N | L | R | E | I | F | L | K | G | I | E | E | G | 308 |
| MOUSE | P | Q | L | P | - | - | E | H | L | K | E | S | F | L | E | A | S | Q | N | G | 381 |
| RAT | P | Q | I | P | - | - | D | H | L | K | E | S | F | L | E | A | C | Q | N | A | 382 |
| YEAST | P | F | I | P | - | - | K | S | L | R | P | S | F | D | E | A | V | S | Q | G | 310 |

# FIG. 1D



| | | | | | | | | | | 430 | | | | | | | | | | 440 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | - | S | H | I | K | I | T | Q | A | K | R | I | P | A | T | L | S | R | K | K | 330 |
| B. NAPUS 411 | - | A | Q | I | K | A | M | P | T | K | R | M | E | A | T | L | S | E | K | Q | 329 |
| ARABIDOPSIS | L | P | E | I | K | S | T | A | T | K | S | M | S | S | R | L | C | D | K | R | 328 |
| MOUSE | - | - | R | L | R | T | M | P | A | S | F | L | P | P | S | S | V | N | K | R | 399 |
| RAT | - | - | R | L | R | T | M | P | A | S | F | L | P | P | S | S | V | N | K | R | 400 |
| YEAST | - | - | K | F | R | A | M | P | N | S | Y | L | P | A | R | Q | N | D | V | T | 328 |

| | | | | | | | | | | 450 | | | | | | | | | | 460 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | G | V | I | V | L | G | D | A | F | N | M | R | H | P | V | I | A | S | G | M | 350 |
| B. NAPUS 411 | G | V | I | V | L | G | D | A | F | N | M | R | H | P | A | I | A | S | G | M | 349 |
| ARABIDOPSIS | G | V | I | V | L | G | D | A | F | N | M | R | H | P | I | I | A | S | G | M | 348 |
| MOUSE | G | V | L | I | L | G | D | A | Y | N | L | R | H | P | L | T | G | G | G | M | 419 |
| RAT | G | V | L | L | L | G | D | A | Y | N | L | R | H | P | L | T | G | G | G | M | 420 |
| YEAST | G | M | C | V | I | G | D | A | L | N | M | R | H | P | L | T | G | G | G | M | 348 |

| | | | | | | | | | | 470 | | | | | | | | | | 480 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | M | V | L | L | S | D | I | L | I | L | S | R | L | L | K | P | L | G | N | L | 370 |
| B. NAPUS 411 | M | V | V | L | S | D | I | L | I | L | R | R | L | L | Q | P | L | R | N | L | 369 |
| ARABIDOPSIS | M | V | A | L | S | D | I | C | I | L | R | N | L | L | K | P | L | P | N | L | 368 |
| MOUSE | T | V | A | L | K | D | I | K | L | W | R | Q | L | L | K | D | I | P | D | L | 439 |
| RAT | T | V | A | L | K | D | I | K | I | W | R | Q | L | L | K | D | I | P | D | L | 440 |
| YEAST | T | V | G | L | H | D | V | V | L | L | I | K | K | I | G | D | L | - | D | F | 367 |

| | | | | | | | | | | 490 | | | | | | | | | | 500 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | G | D | E | N | K | V | S | E | V | M | K | S | F | Y | A | L | R | K | P | M | 390 |
| B. NAPUS 411 | S | D | A | N | K | V | S | E | V | I | K | S | F | Y | V | I | R | K | P | M | 389 |
| ARABIDOPSIS | S | N | T | K | K | V | S | D | L | V | K | S | F | Y | I | I | R | K | P | M | 388 |
| MOUSE | Y | D | D | A | A | I | F | Q | A | K | K | S | F | F | W | S | R | K | R | T | 459 |
| RAT | Y | D | D | A | A | I | F | Q | A | K | K | S | F | F | W | S | R | K | R | S | 460 |
| YEAST | S | D | R | E | K | V | L | D | E | L | L | D | Y | H | F | E | R | K | S | Y | 387 |

| | | | | | | | | | | 510 | | | | | | | | | | 520 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | S | A | T | V | N | T | L | G | N | S | F | W | Q | V | L | I | A | S | T | D | 410 |
| B. NAPUS 411 | S | A | T | V | N | T | L | G | N | A | F | S | Q | V | L | I | A | S | T | D | 409 |
| ARABIDOPSIS | S | A | T | V | N | T | L | A | S | I | F | S | Q | V | L | V | A | T | T | D | 408 |
| MOUSE | H | S | F | V | V | N | V | L | A | Q | A | L | Y | E | L | F | S | A | T | D | 479 |
| RAT | H | S | F | V | V | N | V | L | A | Q | A | L | Y | E | L | F | S | A | T | D | 480 |
| YEAST | D | S | - | V | I | N | V | L | S | V | A | L | Y | S | L | F | A | A | D | S | 406 |

| | | | | | | | | | | 530 | | | | | | | | | | 540 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | E | A | K | E | A | M | R | Q | G | C | F | D | Y | L | S | S | G | G | F | R | 430 |
| B. NAPUS 411 | E | A | K | E | A | M | R | Q | G | C | F | D | Y | L | S | S | G | G | F | R | 429 |
| ARABIDOPSIS | E | A | R | E | G | M | R | Q | G | C | F | N | Y | L | A | R | G | D | F | K | 428 |
| MOUSE | D | S | L | H | Q | L | R | K | A | C | F | L | Y | F | K | L | G | G | E | C | 499 |
| RAT | D | S | L | R | Q | L | R | K | A | C | F | L | Y | F | K | L | G | G | E | C | 500 |
| YEAST | D | N | L | K | A | L | Q | K | G | C | F | K | Y | F | Q | R | G | G | D | C | 426 |

| | | | | | | | | | | 550 | | | | | | | | | | 560 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | T | S | G | L | M | A | L | I | G | G | M | N | P | R | P | L | S | L | F | Y | 450 |
| B. NAPUS 411 | T | S | G | M | M | A | L | L | G | G | M | N | P | R | P | L | S | L | I | F | 449 |
| ARABIDOPSIS | T | R | G | L | M | T | I | L | G | G | M | N | P | H | P | L | T | L | V | L | 448 |
| MOUSE | V | T | G | P | V | G | L | L | S | I | L | S | P | H | P | L | V | L | I | R | 519 |
| RAT | L | T | G | P | V | G | L | L | S | I | L | S | P | D | P | L | L | L | I | R | 520 |
| YEAST | V | N | K | P | V | E | F | L | S | G | V | L | P | K | P | L | Q | L | T | R | 446 |

| | | | | | | | | | | | 570 | | | | | | | | | 580 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | H | L | F | V | I | S | L | S | S | I | G | Q | L | L | S | P | F | P | T | P | 470 |
| B. NAPUS 411 | H | L | C | G | I | T | L | S | S | I | G | Q | L | L | S | P | F | P | S | P | 469 |
| ARABIDOPSIS | H | L | V | A | I | T | L | T | S | M | G | H | L | L | S | P | F | P | S | P | 468 |
| MOUSE | H | F | F | S | V | A | I | Y | A | T | - | - | - | - | - | - | - | - | - | - | 530 |
| RAT | H | F | F | S | V | A | V | Y | A | T | - | - | - | - | - | - | - | - | - | - | 531 |
| YEAST | V | F | F | A | V | A | F | Y | T | I | - | - | - | - | - | - | - | - | - | - | 457 |

| | | | | | | | | | | | 590 | | | | | | | | | 600 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | L | R | V | W | H | S | L | R | L | L | D | L | S | L | K | M | L | V | P | H | 490 |
| B. NAPUS 411 | L | G | I | W | H | S | L | R | L | F | - | - | - | - | - | - | - | - | - | - | 480 |
| ARABIDOPSIS | R | R | F | W | H | S | L | R | I | L | A | W | A | L | Q | M | L | G | A | H | 488 |
| MOUSE | - | - | - | - | - | Y | F | C | F | K | S | E | P | W | A | T | K | P | R | A | 544 |
| RAT | - | - | - | - | - | Y | F | C | F | K | S | E | P | W | A | T | K | P | R | A | 545 |
| YEAST | - | - | - | - | - | Y | L | N | M | E | E | R | G | F | L | G | L | P | M | A | 471 |

| | | | | | | | | | | | 610 | | | | | | | | | 620 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | L | K | A | E | G | I | G | Q | M | L | S | P | T | N | A | A | A | Y | R | K | 510 |
| B. NAPUS 411 | - | - | - | - | G | V | S | Q | M | L | S | P | A | Y | A | A | A | Y | R | K | 495 |
| ARABIDOPSIS | L | V | D | E | G | F | K | E | M | L | I | P | T | N | A | A | A | Y | R | R | 508 |
| MOUSE | L | F | S | S | G | A | V | L | Y | K | A | C | S | I | L | F | P | L | I | Y | 564 |
| RAT | L | F | S | S | G | A | I | L | Y | K | A | C | S | I | I | F | P | L | I | Y | 565 |
| YEAST | L | L | E | G | I | M | I | L | I | T | A | I | R | V | F | T | P | F | L | F | 491 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| B. NAPUS 111 | S | Y | M | A | A | T | V | V | - | 519 |
| B. NAPUS 411 | S | Y | M | T | A | T | A | L | - | 504 |
| ARABIDOPSIS | N | Y | I | A | T | T | T | V | - | 517 |
| MOUSE | S | E | M | K | Y | L | V | H | - | 573 |
| RAT | S | E | M | K | Y | L | V | H | - | 574 |
| YEAST | G | E | - | - | - | L | I | G | - | 497 |

**FIG. 1E**

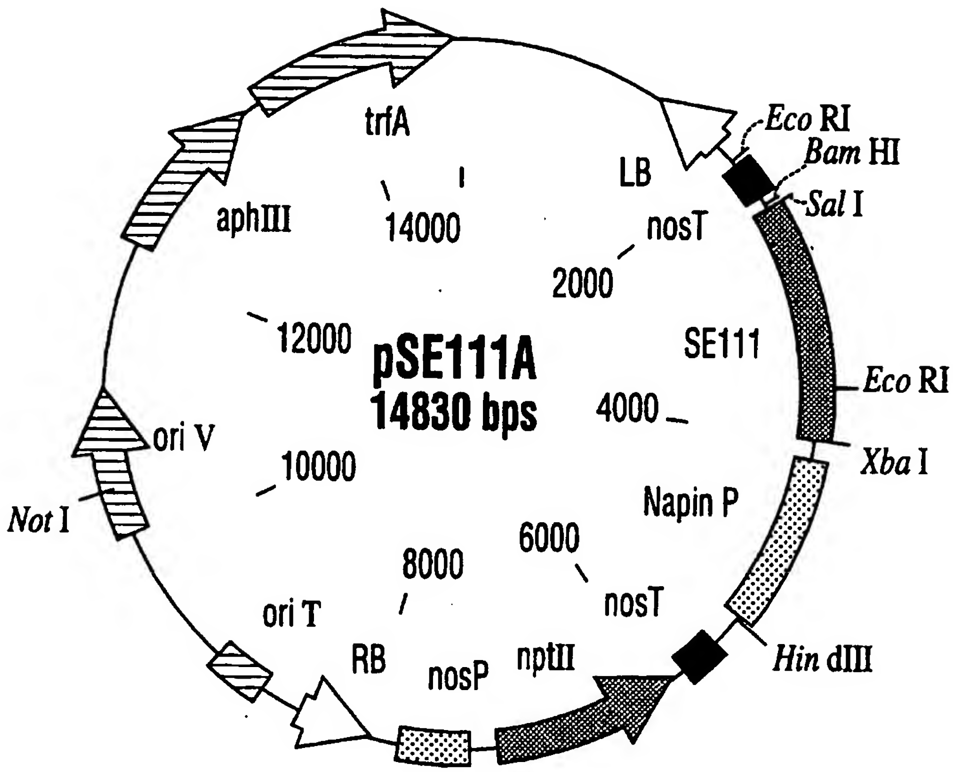

pSE111A
14830 bps
trfA
aphIII
14000
LB
Eco RI
Bam HI
Sal I
nosT
2000
12000
SE111
Eco RI
4000
Xba I
ori V
10000
Napin P
Not I
6000
8000
nosT
ori T
RB
nosP
nptII
Hin dIII

FIG. 2

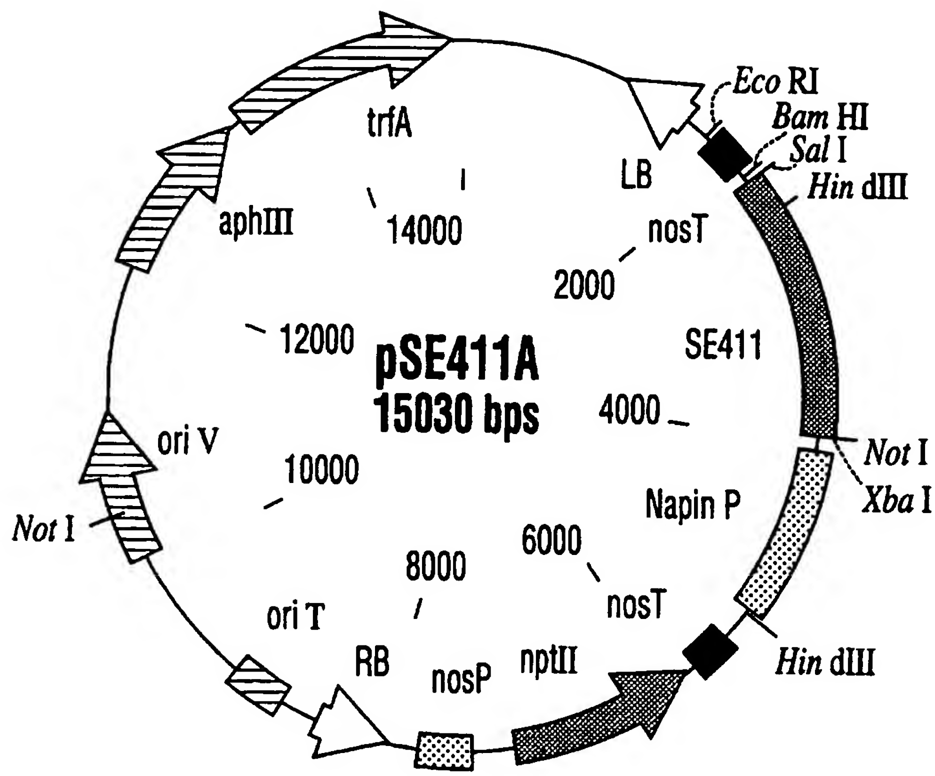
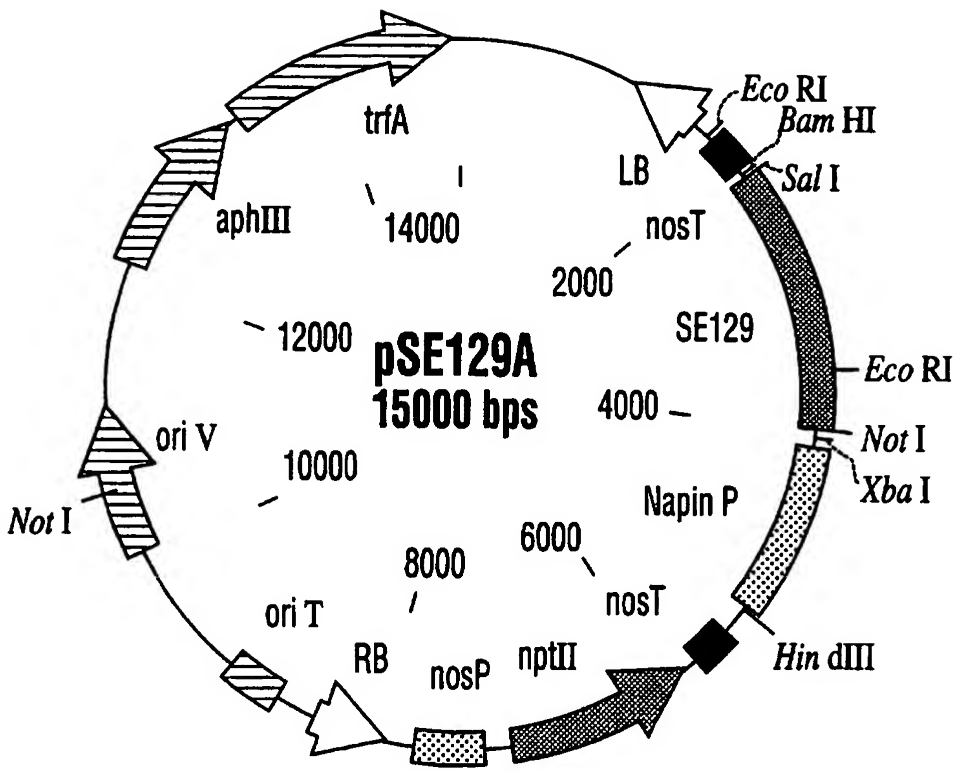

Eco RI
Bam HI
Sal I
Hin dIII
trfA
LB
aphIII
14000
nosT
2000
12000
pSE411A
15030 bps
SE411
4000
ori V
10000
Not I
Xba I
Not I
Napin P
6000
8000
nosT
ori T
RB
nosP
nptII
Hin dIII

FIG. 3



Eco RI
Bam HI
Sal I
trfA
LB
aphIII
14000
nosT
2000
SE129
12000
pSE129A
15000 bps
Eco RI
4000
ori V
Not I
Xba I
10000
Napin P
Not I
6000
8000
nosT
ori T
RB
nosP
nptII
Hin dIII


FIG. 4

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 97/00175

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/53 C12N15/82 C12N9/02 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GENE, vol. 107, no. 1, 1991, pages 155-160, XP002033661 JANDROSITZ A. ET AL.: "The gene encoding squalene epoxidase from Saccharomyces cerevisiae: cloning and characterization" cited in the application | 1,2 |
| A | see the whole document | 3-26 |
| | --- | |
| X | EMBL DATABASE, 7 March 1996, HEIDELBERG, XP002033786 NEWMAN T. ET AL.: "AC N64916" see the whole document | 1-4 |
| | --- | |
| X | EMBL DATABASE, 18 March 1995, HEIDELBERG, XP002033787 DE LOO F. ET AL.: "AC T15019" see the whole document | 1-4 |
| | --- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search: 26 June 1997

Date of mailing of the international search report: 11.07.97

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Fax: (+31-70) 340-3016

Authorized officer
Kania, T

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# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 97/00175

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| --- | --- | --- |
| X | EMBL DATABASE,<br>4 February 1995, HEIDELBERG, XP002033788<br>NEWMAN T. ET AL.: "AC T44667"<br>see the whole document | 1-3,18 |
| A | US 5 349 126 A (CHAPPELL JOSEPH ET AL) 20 September 1994<br>cited in the application<br>* see the whole document, esp. part II B,H * | 5-15,<br>24-26 |
| A | JP 06 090 743 A (MITSUBISHI OIL CO LTD) 5 April 1994<br>* see abstract * | 1-26 |
| P,X | EMBL DATABASE,<br>27 May 1996, HEIDELBERG, XP002033789<br>NEWMAN T. ET AL.: "AC W43353"<br>see the whole document | 1-4 |
| T | WO 96 09393 A (REYNOLDS TECHNOLOGIES INC ;BIOSOURCE TECH INC (US); HANLEY KATHLEE)<br>28 March 1996<br>see the whole document | 1-26 |

1

| Applicant's or agent's file reference number | ..1573-PT. | International application | PCT/CA97/00175. |
|---|---|---|---|

## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

DEPOSIT RECEIPT ATTACHED.

**A.** The indications made below relate to the microorganism referred to in the description on page 24, line 17 - 22.

**B. IDENTIFICATION OF DEPOSIT** — Further deposits are identified on an additional sheet ☐

Name of depositary institution

AMERICAN TYPE CULTURE COLLECTION.

Address of depositary institution *(including postal code and country)*

12301 PARKLAWN DRIVE
ROCKVILLE MD, USA, 20852

| Date of deposit | Accession Number |
|---|---|
| MARCH 5, 1997. | ATCC 97908<br>ATCC 97909<br>ATCC 97910. |

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

MICROORGANISMS TO BE MADE AVAILABLE TO PUBLIC ONLY BY ISSUANCE OF A SAMPLE TO AN EXPERT NOMINATED BY APPLICANT PRIOR TO ISSUANCE OF PATENT OR ABANDONMENT.

**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

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|---|---|---|---|

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**B. IDENTIFICATION OF DEPOSIT** — Further deposits are identified on an additional sheet [X]

Name of depositary institution: AMERICAN TYPE CULTURE COLLECTION.

Address of depositary institution *(including postal code and country)*:
12301 PARKLAWN DRIVE
ROCKVILLE MD, USA, 20852

| Date of deposit | Accession Number |
|---|---|
| JANUARY 9, 1997 | ATCC 97845<br>ATCC 97846<br>ATCC 97847. |

**C. ADDITIONAL INDICATIONS** *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

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**D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE** *(if the indications are not for all designated States)*

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte  nal Application No
PCT/CA 97/00175

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | US 5589619 A | 31-12-96 |
| | | AU 653748 B | 13-10-94 |
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| | | EP 0480730 A | 15-04-92 |
| | | JP 5115298 A | 14-05-93 |
| | | TR 25647 A | 01-07-93 |
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| JP 06090743 A | 05-04-94 | NONE | |
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